AGROECOSYSTEMS FACING GLOBAL CLIMATE CHANGE: THE SEARCH FOR SUSTAINABILITY

EDITED BY: José M. Mirás-Avalos and Philippe C. Baveye
PUBLISHED IN: Frontiers in Plant Science and Frontiers in Ecology and Evolution

frontiers Research Topics

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ISSN 1664-8714
ISBN 978-2-88945-715-1
DOI 10.3389/978-2-88945-715-1

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AGROECOSYSTEMS FACING GLOBAL CLIMATE CHANGE: THE SEARCH FOR SUSTAINABILITY

Topic Editors:
José M. Mirás-Avalos, Universidade de Santiago de Compostela, Spain
Philippe C. Baveye, AgroParisTech, France

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Global change is posing new threats to agroecosystems. First, climate modifications in the spatial and temporal distribution of rainfall increase the risks of severe droughts during the growing season of most crops. Second, conventional agriculture has led to the extension of mono-crop fields that decreased biodiversity in agroecosystems; it is possible that these fields will lack resilience when faced with changing climate. In addition, a new conscience has arisen and consumers tend to look for healthy products that, sometimes, do not match the objectives of conventional agriculture.

In this context, sustainable and environmentally friendly agricultural practices that can cope with the new global change scenario are needed. This eBook compiles state-of-the-art research on the agroecosystems response to global change and on how to manage these new scenarios. Despite the broad scope of the topic, this Research Topic covers a wide range of subjects, including biodiversity, crop performance, novel agricultural practices and soil properties.

Citation: Mirás-Avalos, J. M., Baveye, P. C., eds. (2019). Agroecosystems Facing Global Climate Change: The Search for Sustainability. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-715-1

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frontiers
in Environmental Science

EDITORIAL
published: 12 November 2018
doi: 10.3389/fenvs.2018.00135

Editorial: Agroecosystems Facing Global Climate Change: The Search for Sustainability

José M. Mirás-Avalos[1]* *and Philippe C. Baveye*[2]

[1] *GI-1716, Proyectos y Planificación, Departamento de Ingeniería Agroforestal, Universidade de Santiago de Compostela, Escola Politécnica Superior de Enxeñaría, Lugo, Spain,* [2] *ECOSYS Unit, AgroParisTech, Université Paris-Saclay, Thiverval-Grignon, France*

Keywords: agroecology, best management practices, biotic and abiotic stresses, environmental sustainability, native plants

OPEN ACCESS

Edited by:
Vimala D. Nair,
University of Florida, United States

Reviewed by:
B. Mohan Kumar,
Nalanda University, India
Sotirios Archontoulis,
Iowa State University, United States

Correspondence:
José M. Mirás-Avalos
jmirasa@udc.es

Specialty section:
This article was submitted to Agroecology and Ecosystem Services, a section of the journal Frontiers in Environmental Science

Received: *14 September 2018*
Accepted: *24 October 2018*
Published: *12 November 2018*

Citation:
Mirás-Avalos JM and Baveye PC (2018) Editorial: Agroecosystems Facing Global Climate Change: The Search for Sustainability. Front. Environ. Sci. 6:135. doi: 10.3389/fenvs.2018.00135

Editorial on the Research Topic

Agroecosystems Facing Global Climate Change: The Search for Sustainability

Climate change and variability in years to come should in principle affect agroecosystems worldwide due to impacts on plant growth and yield by elevated atmospheric CO_2 concentration, higher temperatures, altered precipitation regimes, and increased frequency of extreme events, as well as modified weed, pest, and pathogen pressure (Altieri et al., 2015). In addition, because the diversity of agricultural systems has been reduced to maximize mono-crop yields under favorable conditions, it is possible that these systems will lack resilience when faced with changing climate (Isbell, 2015). These future prospects have prompted a new conscience about environmentally friendly agroecosystems, and policies are being actively promoted, which aim to prohibit or at least limit pesticide use, as well as promote the adoption of best management practices (Lamichhane et al., 2016). Furthermore, consumers tend to shift to healthy products, away, sometimes, from less healthy ones resulting from industrialized agriculture (Sogari et al., 2016). In this context, researchers have endeavored to find and establish the best options that farmers could adopt to preserve natural resources such as soil and water while maintaining the yields and economic benefits of traditional practices (Fleming and Vanclay, 2010; Iglesias and Garrote, 2015; van der Laan et al., 2017).

In this general context, this Research Topic aims to present recent scientific progress concerning agricultural practices that allow agroecosystems to cope with the new challenges imposed by global change. The Research Topic comprises 11 articles, including 6 Original Research articles, 2 Reviews, 2 Perspective articles, and 1 Method article. No doubt there are many more issues that could fit under the very broad scope of the Research Topic, but the articles gathered already cover a sizeable range of subjects, from novel agricultural practices to biodiversity, crop performance, and soil properties.

Soil is a non-renewable resource that deserves special attention in the context of sustainable agriculture under climate change. In this Research Topic, two articles focus on this important resource. Gao et al. study the effects that afforestation may have on soil inorganic carbon (SIC) sequestration in Northwest China. This form of carbon is the dominant one in arid and semiarid areas; therefore, a subtle fluctuation of SIC pool can alter the regional carbon budget. These authors found that the SIC pool increased after afforestation for 30 years, doubling the SIC amount observed in sandy soils, indicating the high potential of afforestation for sequestering carbon. In addition, Bhat et al. compare the soil biological activity, focusing on phosphorus availability for crops, under long-term organic management vs. conventional agriculture in central India. They reported that organic systems possessed equal capabilities of supplying phosphorus for crop growth as conventional systems due to a higher biological activity.

An interesting perspective article by Nair et al. highlights the potentialities and limitations that biochar application has for sustainable agriculture. Over the last decade, many authors have promoted the idea that applying biochar or agrichar to soils presents a number of possible benefits, among which are the reduction of bulk density, enhancement of water-holding capacity, and stabilization of organic matter. Nevertheless, the merits of biochar remain extremely controversial (e.g., Sánchez-García et al., 2014; Baveye et al., 2018). In that respect, Nair et al. point out that several problems and bottlenecks remain to be addressed before one could consider widespread production and use of biochar. The current state of knowledge is based largely on limited small-scale studies under laboratory and greenhouse conditions. Properties of biochar vary with both the feedstock from which it is produced and the method of production. The availability of feedstock as well as the economic merits, energy needs, and potential environmental risks of its large-scale production and use remain to be investigated. Nevertheless, Nair et al. argue in favor of the viewpoint that biochar could play a significant role in facing the challenges posed by climate change and threats to agroecosystem sustainability.

The reduction of diversity in agroecosystems in a climate change context is the subject of three articles within this Research Topic. First, Nair et al. highlights the virtues of multi-strata tree + crop (MTC) systems. These systems are based on niche complementarity among species. This implies that MTC systems are structurally and functionally more complex than crop or tree monocultures, resulting in greater efficiency for capturing and using resources (light, water, nutrients). Ecosystem services, future scenarios and directions of MTC systems are clearly described in this thought-provoking article. Second, Shelef et al. review the value of native plants and local production as a means to promote food diversity and agricultural resilience. These authors used the example of producing pine nuts in the Western United States to illustrate their proposal to support local food production in an ecologically sustainable manner. Third, Chedraoui et al. review in detail the literature devoted to *Capparis spinosa* (L.), a xerophilous species with a broad range of benefits and potentialities for agriculture in Eastern Mediterranean countries. This review provides information about the origin, distribution, taxonomy, genetics, cultivation, phytochemical composition of this species, as well as some of its traditional uses. Along this line of preserving biodiversity, Arheimer et al. are concerned with the decrease of snowy periods in northern Europe, which could lead to diversity losses in riparian mixed forests that are flooded during some periods of the year. These authors propose, through a modeling approach, to use artificial floods to preserve diversity in these ecosystems; however, several factors, both technical and economic, restrict the practical implementation of this proposal.

Crop performance under different conditions has been addressed in two articles within this Research Topic. First, Li et al. are interested in assessing the extent of soybean nitrogen fixation under elevated CO_2 conditions, since these could limit crop performance due to nitrogen limitations. These conditions increase the ability of plants to take up nitrogen by facilitating root proliferation and nodule growth. Second, the use of marginal lands for growing sorghum for bioethanol production is the subject of the article by Tang et al. They conclude that energy sorghum grown on marginal lands has a very low potential for ethanol production and, therefore, offers a lower possibility for commercial feedstock supply when compared to that grown on regular croplands. However, screening suitable varieties may improve the growth of sorghum and its chemical properties for ethanol production on marginal lands.

From an economic perspective, Riar et al. present a diagnosis of biophysical and socio-economic factors influencing the choice to adopt organic or conventional systems for cotton production. Organic farmers are motivated by the sustainability of cotton production and growing food without pesticides, whereas conventional farmers are sensitive to their reputation in the community.

Finally, in an interesting methodological article, Kundel et al. explain the design and the advantages of a new model of rainout-shelters for climate change experiments in agroecosystems. These devices prove able to sustain heavy weather and could be used in agricultural fields where management operations require the removal of the rainout-shelters. Moreover, they prevent common artifacts that occur when one uses other devices.

Clearly, the 11 articles composing this Research Topic only begin to scratch the surface of a very broad area of research (as noted by the absence of articles devoted to soil organic carbon), which will undoubtedly become the focus of increasing attention, as time goes by and the effects of global climate change on agroecosystems become more pronounced and noticeable. In this context, it is our hope that this Research Topic will contribute in some measure to fostering a healthy debate on whither the research should be heading in years to come.

AUTHOR CONTRIBUTIONS

JM-A conceived and coordinated this Research Topic. JM-A and PCB read the various articles included in the RT, contributed to the writing of this editorial, and jointly approved it.

ACKNOWLEDGMENTS

The editors want to express their profound gratitude to all the reviewers for their valuable contributions, which helped to achieve high standards for the contributed papers.

REFERENCES

Altieri, M. A., Nicholls, C. I., Henao, A., and Lana, M. A. (2015). Agroecology and the design of climate change-resilient farming systems. *Agron. Sustain. Dev.* 35, 869–890. doi: 10.1007/s13593-015-285-2

Baveye, P. C., Berthelin, J., Tessier, D., and Lemaire, G. (2018). The "4 per 1000" initiative: a credibility issue for the soil science community? *Geoderma* 309(Suppl. C), 118–123. doi: 10.1016/j.geoderma.2017.05.005

Fleming, A., and Vanclay, F. (2010). Farmer responses to climate change and sustainable agriculture. a review.

Agron. Sustain. Dev. 30, 11–19. doi: 10.1051/agro/2009028

Iglesias, A., and Garrote, L. (2015). Adaptation strategies for agricultural water management under climate change in Europe. *Agric. Water Manage.* 155, 113–124. doi: 10.1016/j.agwat.2015.03.014

Isbell, F. (2015). Agroecosystem diversification. *Nat. Plants* 1:15041. doi: 10.1038/NPLANTS.2015.41

Lamichhane, J. R., Dachbordt-Saaydeh, S., Kudsk, P., and Messéan, A. (2016). Toward a reduced reliance on conventional pesticides in European agriculture. *Plant Dis.* 100, 10–24. doi: 10.1094/PDIS-05-15-0574-FE

Sánchez-García, M., Roig, A., Sánchez-Monedero, M. A., and Cayuela, M. L. (2014). Biochar increases soil N_2O emissions produced by nitrification-mediated pathways. *Front. Environ. Sci.* 2:25. doi: 10.3389/fenvs.2014.00025

Sogari, G., Mora, C., and Menozzi, D. (2016). Factors driving sustainable choice: the case of wine. *Br. Food J.* 118, 632–646. doi: 10.1108/BFJ-04-2015-0131

van der Laan, M., Bristow, K. L., Stirzaker, R. J., and Annandale, J. G. (2017). Towards ecologically sustainable crop production: a South African perspective. *Agric. Ecosys. Environ.* 236, 108–119. doi: 10.1016/j.agee.2016.11.014

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Plant Science

ORIGINAL RESEARCH
published: 19 July 2017
doi: 10.3389/fpls.2017.01282

Soil Inorganic Carbon Sequestration Following Afforestation Is Probably Induced by Pedogenic Carbonate Formation in Northwest China

*Yang Gao[1,2], Jing Tian[2], Yue Pang[2] and Jiabin Liu[1,3]**

[1] State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling, China, [2] College of Forestry, Northwest A&F University, Yangling, China, [3] College of Natural Resources and Environment, Northwest A&F University, Yangling, China

In arid and semiarid areas, the effects of afforestation on soil organic carbon (SOC) have received considerable attention. In these areas, in fact, soil inorganic carbon (SIC), rather than SOC, is the dominant form of carbon, with a reservoir approximately 2–10 times larger than that of SOC. A subtle fluctuation of SIC pool can strongly alter the regional carbon budget. However, few studies have focused on the variations in SIC, or have used stable soil carbon isotopes to analyze the reason for SIC variations following afforestation in degraded semiarid lands. In the Mu Us Desert, northwest China, we selected a shifting sand land (SL) and three nearby forestlands (*Populus alba*) with ages of 8 (P-8), 20 (P-20) and 30 (P-30) years, and measured SIC, SOC, soil organic and inorganic δ^{13}C values (δ^{13}C-SOC and δ^{13}C-SIC) and other soil properties. The results showed that SIC stock at 0–100 cm in SL was 34.2 Mg ha^{-1}, and it increased significantly to 42.5, 49.2, and 68.3 Mg ha^{-1} in P-8, P-20, and P-30 lands, respectively. Both δ^{13}C-SIC and δ^{13}C-SOC within the 0–100 cm soil layer in the three forestlands were more negative than those in SL, and gradually decreased with plantation age. Afforestation elevated soil fine particles only at a depth of 0–40 cm. The entire dataset (260 soil samples) exhibited a negative correlation between δ^{13}C-SIC and SIC content ($R^2 = 0.71$, $P < 0.01$), whereas it showed positive correlation between SOC content and SIC content ($R^2 = 0.52$, $P < 0.01$) and between δ^{13}C-SOC and δ^{13}C-SIC ($R^2 = 0.63$, $P < 0.01$). However, no correlation was observed between SIC content and soil fine particles. The results indicated that afforestation on shifting SL has a high potential to sequester SIC in degraded semiarid regions. The contribution of soil fine particle deposition by canopy to SIC sequestration is limited. The SIC sequestration following afforestation is very probably caused by pedogenic carbonate formation, which is closely related to SOC accumulation. Our findings suggest that SIC plays an important role in the carbon cycle in semiarid areas and that overlooking this carbon pool may substantially lead to underestimating carbon sequestration capacity following vegetation rehabilitation.

Keywords: afforestation, degraded semiarid regions, pedogenic inorganic carbon, soil inorganic carbon, stable carbon isotope

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Edited by:
José Manuel Mirás-Avalos,
Centro de Edafología y Biología
Aplicada del Segura (CSIC), Spain

Reviewed by:
Rui Liu,
University of Melbourne, Australia
Ichiro Tayasu,
Research Institute for Humanity
and Nature, Japan

***Correspondence:**
Jiabin Liu
liujb@nwsuaf.edu.cn

Specialty section:
This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Plant Science

Received: *06 April 2017*
Accepted: *07 July 2017*
Published: *19 July 2017*

Citation:
Gao Y, Tian J, Pang Y and Liu J
(2017) Soil Inorganic Carbon
Sequestration Following Afforestation
Is Probably Induced by Pedogenic
Carbonate Formation in Northwest
China. Front. Plant Sci. 8:1282.
doi: 10.3389/fpls.2017.01282

INTRODUCTION

Arid and semiarid areas cover approximately 41% of the Earth's land surface (Reynolds et al., 2007; Delgado-Baquerizo et al., 2013). In these areas, desertification is an extremely challenging environmental problem leading to serious land degradation and enormous losses of soil carbon (Lal, 2009; Li et al., 2015). However, if appropriate restoration measures can be successfully implemented on degraded lands, it is possible to effectively curb land degradation and substantially improve the soil properties in these lands (Lal, 2004; Huang et al., 2012). Afforestation is an important restoration measure for degraded lands and is generally considered to have great potential to combat desertification, protect soils and alter the soil carbon pool (Lal, 2010). The soil carbon pool comprises the soil organic carbon (SOC) and soil inorganic carbon (SIC) pools (Zhang et al., 2015). Because of its potentially rapid response to afforestation, the SOC pool has received considerable attention and has been extensively investigated (Jackson et al., 2002; Deng et al., 2014). In contrast to the great progress made in understanding the dynamics of the SOC pool, the effects of afforestation on the SIC pool have received relatively less consideration (Wang et al., 2010; Meyer et al., 2014). In fact, SIC, rather than SOC, is the dominant form of carbon in arid and semiarid areas (Mielnick et al., 2005; Mi et al., 2008), with a reservoir approximately 2–10 times larger than that of SOC (Schlesinger, 1982; Tan et al., 2014). Due to the large reservoir of SIC, a subtle fluctuation in the SIC pool will strongly alter the carbon budget in arid and semiarid areas (Landi et al., 2003; Jin et al., 2014). It is therefore important to have a thorough understanding of the dynamics of SIC pool following afforestation in these regions.

Changes in SIC following afforestation in arid and semiarid areas exhibit contrasting trends, some of which are in direct opposition. For instance, in the Horqin Sandy Land and Badain Jaran Desert, China, planting Mongolian pine and poplar significantly stimulated the accumulation of SIC (Su et al., 2010; Li Y.Q. et al., 2013). In contrast, in the Columbia Plateau of Oregon, United States, poplar afforestation was found to reduce the SIC stock (Sartori et al., 2007). Another study in the Loess Plateau of China reported that afforestation simply redistributed SIC along the soil profile without affecting its total quantity (Chang et al., 2012). These results indicate that the effects of afforestation on SIC stock need to be further examined in arid and semiarid areas.

Importantly, uncertainty nonetheless remains as to why SIC showed variation following afforestation. There are several geological methods (such as scanning electron microscopes) for studying SIC variations (Zamanian et al., 2016). Among these, stable soil carbon isotopes (^{13}C) have been demonstrated to be an applicable and crucial indicator revealing the reason for SIC variations following land use changes (Cerling et al., 1989; Stevenson et al., 2005). The SIC pool consists of lithogenic inorganic carbon (LIC) and pedogenic inorganic carbon (PIC) pools, and these two subpools have different δ^{13}C values (Jobbágy and Jackson, 2003; Chang et al., 2012; Tan et al., 2014). The LIC subpool is inherited from the parent material and generally has high δ^{13}C values (close to zero), whereas the PIC subpool is generated from the precipitation of carbonate ions and generally shows low δ^{13}C values (negative) (Wang et al., 2016; Zamanian et al., 2016). The dynamics of the SIC pool following land use changes are dominated by the LIC and PIC subpools. Various processes in SIC variations, including the mixing of LIC with PIC and the reaction of soil carbonate with biogenic CO_2, can be sensitively and precisely reflected in δ^{13}C values (Stevenson et al., 2005; Monger et al., 2015). The use of stable soil carbon isotopes method, in which the soil inorganic δ^{13}C value (δ^{13}C-SIC) and the soil organic δ^{13}C value (δ^{13}C-SOC) are measured, has been found to be an ideal approach to studying the inherent mechanisms of SIC dissolution, sequestration and transformation following land use changes (Stevenson et al., 2005; Rao et al., 2006; Li G.J. et al., 2013; Wang J.P. et al., 2015). In arid croplands, determining the changes in δ^{13}C-SIC and δ^{13}C-SOC following straw organic amendments, revealed that such amendments enhanced PIC formation and led to SIC accumulation (Wang et al., 2014; Wang X.J. et al., 2015). In semiarid restored grassland, a decrease in δ^{13}C-SIC indicated that soil carbonate exchanged with biogenic CO_2, resulting in lower SIC stock in grassland than in farmland (Liu et al., 2014). Despite the value provided by the existing carbon isotope methods, they have not been extensively utilized to explore the reason for SIC variations after afforestation in degraded semiarid lands, particularly for afforestation on shifting sand land (SL).

Sand land, which is widely distributed in northwest China, is characterized by extreme deterioration of the plant and soil environment. Afforestation and shrub-planting are commonly suggested as options to combat desertification (Zhang K. et al., 2010; Zhang Y. et al., 2013). Previous studies have conclusively demonstrated that afforestation on SL significantly promotes SOC storage (Liu et al., 2013; Li et al., 2016). However, few studies have focused on the variations in SIC, or have used stable soil carbon isotopes to analyze the mechanisms underlying SIC variations following afforestation on SL. The use of the related field data along a chronosequence of afforestation, which could more precisely and reliably determine the dynamics of SIC, has rarely been reported. The changes in soil carbon along a chronosequence of afforestation are often studied by comparing the different-aged forestlands within a designated area (space-for-time substitution approach) (Farley et al., 2004; Qiu et al., 2015), as the historical data in a same forestland since the beginning of afforestation cannot be obtained at present. In view of the above deficiencies, we selected an SL and three nearby forestlands (*Populus alba*) with ages of 8 (P-8), 20 (P-20), and 30 (P-30) years within 2 km^2 in the Mu Us Desert, northwest China. We measured SIC, SOC, δ^{13}C-SOC and δ^{13}C-SIC in both the SL and the three different-aged forestlands at depth of 100 cm. The objectives of this research were (1) to examine the changes in SIC along a chronosequence of afforestation and (2) to explore the reasons for SIC variations following afforestation using the carbon isotope method.

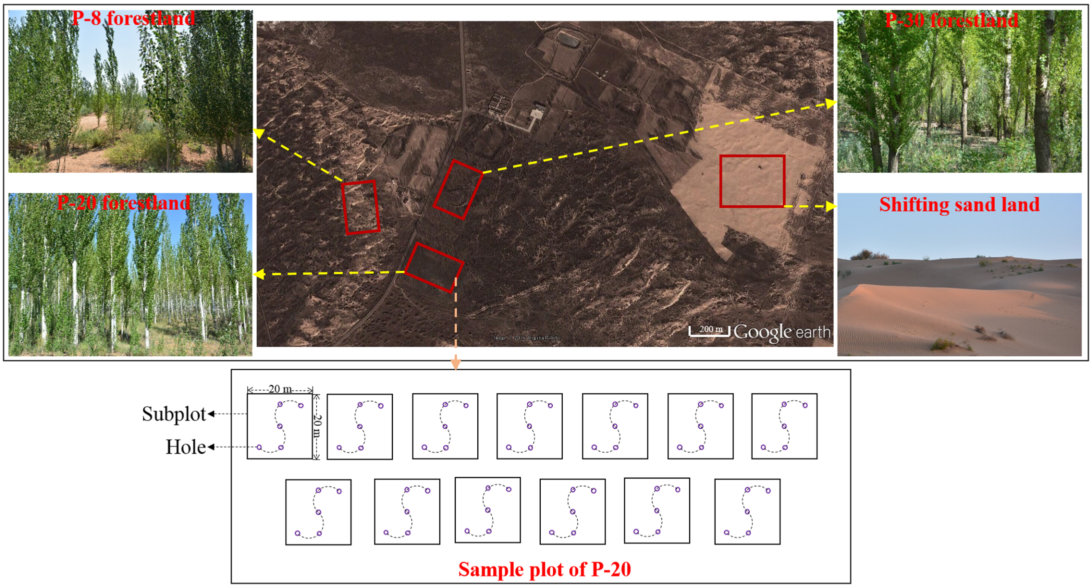

FIGURE 1 | Distribution of the four sample plots in the study area and the relationships between sample plot, subplot and hole. The satellite image was obtained from Google Earth and was taken in January 2013. Other pictures were taken by YG in September 2015.

MATERIALS AND METHODS

Study Site Description

The study site is located at the Station of Chunlan Bai Desertification Control, Yanchi County, Ningxia Province, China (107°27′ E, 37°54′ N), on the southwestern edge of the Mu Us Desert. The region has a typical temperate continental monsoon climate with an elevation of 1308 m. The mean annual precipitation is 275 mm, with 73% occurring in summer and autumn. The mean annual temperature is 7°C. The average relative humidity is 51% and the frost-free period lasts for 128 days. According to the US Soil Taxonomy system, the soil type is quartisamment (Gao et al., 2014), with a pH range of 8.0 to 9.0. In the 1980s, the landscape of the research area was dominated by SL, which comprised many connected active sand dunes devoid of any vegetation. At that time, the groundwater level was high enough (2 m) to supply water for tree growth. Afforestation with poplar (*Populus alba*) on SL was successively performed by Chunlan Bai and her family to restrict sand movement and to protect their homeland. At present, forestlands with different plantation ages have been established at the study site. Additionally, areas of SL at some distance from human habitation have not been managed, and have remained active. Previous studies have confirmed that the soil properties in the SL do not vary over a prolonged period of time (Su and Zhao, 2003; Su et al., 2010), suggesting that the soil properties prior to the start of the experiments can be represented by those in the SL at the time of the study. Therefore, the present-day SL can be used as a control for investigating the changes in SIC and soil stable carbon isotopes following afforestation. In this study, we used different-aged forestlands to explore the dynamics of SIC along a chronosequence of afforestation, because there had been no related study in this region and there was a lack of historical data. Within the scope of the 2 km × 1 km in the study site, we selected an SL and its nearby three different-aged forestlands as the four treatments: (1) the SL (control), (2) an 8-year-old poplar land, (3) a 20-year-old poplar land, and (4) a 30-year-old poplar land. For each treatment, we selected one sample plot. The distribution of the four sample plots within the study site is illustrated in **Figure 1**, and information on the four sample plots is presented in **Table 1**.

Soil Sampling and Analyses

Thirteen 20 m × 20 m subplots were randomly selected within each sample plot for soil sampling. In each subplot, five holes (100 cm in depth) along an S-shaped curve were drilled using a soil auger (10 cm in diameter) after removing litter (the relationships between sample plot, subplot and hole are shown in **Figure 1**). The soil samples were obtained at a depth interval of 20 cm from 0 to 100 cm. In each subplot, five soil samples obtained from five holes at the same layer were mixed into a composite sample (approximately 500 g), and five composite samples were achieved at a depth interval of 20 cm from 0 to 100 cm within each subplot. Sixty-five composite samples from the 13 subplots within each sample plot were obtained. After the samples were air-dried, roots were removed from all the 260 composite samples from the four sample plots. For each air-dried composite sample, approximately 50 g soil was taken and retained for measuring particle size distribution using a particle size analyzer (Malven Laser Mastersizer 2000, England). The remaining air-dried composite samples were fully ground in an agate mortar and passed through a 0.1 mm sieve for SIC content, SOC content and soil $\delta^{13}C$ analyses.

TABLE 1 | Characteristics of the four sample plots (mean ± standard deviation; *n* = 13).

Plots	Unit	SL	P-8	P-20	P-30
Sample plot area	ha	4	3	3	3
Plant species		–	*Populus alba*	*Populus alba*	*Populus alba*
Density	trees ha^{-1}	0	585	543	502
Height	m	0	6.4 ± 1.1	12.8 ± 2.2	15.5 ± 2.7
Diameter at breast height	cm	0	6.2 ± 0.8	15.8 ± 1.9	22.6 ± 2.7
Coverage	%	0	28.9	35.7	40.2
Soil electrical conductivity	dS m^{-1}	4.52 ± 0.37	4.73 ± 0.26	4.68 ± 0.24	4.86 ± 0.38
Ca^{2+} in soil	cmol kg^{-1}	4.79 ± 0.24	4.68 ± 0.35	4.92 ± 0.21	5.01 ± 0.18
Mg^{2+} in soil	cmol kg^{-1}	0.33 ± 0.08	0.41 ± 0.04	0.39 ± 0.05	0.48 ± 0.05
Soil total porosity	%	40.3 ± 0.8	43.5 ± 0.7	44.6 ± 0.5	45.7 ± 0.7

After obtaining the 260 composite samples, a soil profile at 0–100 cm was excavated within each subplot. A metal corer (100 cm^3 in volume) was driven into the soil at a depth interval of 20 cm from 0 to 100 cm, and then soil samples were oven dried at 115°C for 24 h and weighed to determine bulk density. From the excavated soil profile in each subplot, additional soil samples were obtained at a depth interval of 20 cm from 0 to 100 cm for measuring Soil pH, using a 2.5:1 ratio of deionized water/soil mass. SOC content was determined using the dichromate oxidation procedure described by Walkley and Black (1934). SIC content was determined using the pressure calcimeter method (Wang et al., 2012). The stocks of SIC were calculated as follows:

$$M = 0.1 \times D \times B \times Z \times ((100 - G)/100) \quad (1)$$

where M is soil carbon stock per unit area (Mg ha^{-1}); D is soil depth (cm); B is bulk density (g cm^{-3}); Z is carbon content (g kg^{-1}) and G is the relative amount of gravel (%). The gravel content was 0 because there was no gravel in the soil.

The detailed methods for determining δ^{13}C-SOC and δ^{13}C-SIC have been described previously by Jin et al. (2014). For the determination of δ^{13}C-SOC, 5 g of ground and sieved soil was steeped in 2 M HCl for 24 h to remove SIC. The treated soil was then washed with distilled water until the pH exceeded 5, and was subsequently dried at 40°C. From each dried soil sample, approximately 30 mg soil was packed in a tin cup and analyzed with an elemental analyzer (Flash EA 1112, Thermo Fisher Scientific, Inc.) and an isotope ratio mass spectrometer (IRMS) (Finnigan MAT Delta plus XP, Thermo Fisher Scientific, Inc.). The contents of the tin cup were combusted at 1000°C in the EA, and then the SOC of the sample in the tin cup was converted to CO_2. The CO_2 from the EA was ionized and its δ^{13}C value was measured by IRMS. The working standards used for determining δ^{13}C-SOC were Protein (Elemental Analyses, Inc., Beijing, China, −26.98‰) and NBS-19 (National Institute of Standards and Technology, Gaithersburg, MD, United States; +1.95‰).

To determine ^{13}C-SIC, approximately 100 mg sieved soil was reacted with 5 mL 100% H_3PO_4 for 2 h at 75°C in a 12 mL sealed vessel of Gas Bench II (Thermo Fisher Scientific, Inc.) to generate CO_2, and the generated CO_2 was measured by IRMS (Finnigan MAT Delta plus XP, Thermo Fisher Scientific, Inc.). The working standards used for determining δ^{13}C-SIC were NBS-18 (National Institute of Standards and Technology, Gaithersburg, MD, United States; −5.01‰) and NBS-19.

The stable isotope compositions of the SOC and SIC, expressed in delta (δ) notation, were both calculated as follows (Coplen, 2011):

$$\delta^{13}C = \frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{standard}} - 1 \quad (2)$$

where $(^{13}C/^{12}C)_{sample}$ and $(^{13}C/^{12}C)_{standard}$ are the atomic ratio of ^{13}C to ^{12}C in the sample and in the Vienna Pee Dee Belemnite (VPDB) standard, respectively. All samples were measured in triplicate. In the three measurements for each sample, the standard deviation of the reported δ^{13}C-SOC and δ^{13}C-SIC in this study was within 0.4 and 0.3‰, respectively.

Statistical Analyses

Statistical analyses were performed using version 16.0 of the SPSS software (SPSS, Chicago, IL, United States). Two-way analysis of variance was conducted to test the effects of soil depth and plant age, as well as their interactions with soil carbon contents and soil δ^{13}C values (**Table 2**). Multiple comparisons and one-way analysis of variance procedures were used to compare the differences in soil carbon contents and soil δ^{13}C values between different treatments within the same depth, and between different soil depths within the same treatment. Mean comparisons were performed using the least-significant-difference test. Linear regression analyses were carried out to evaluate the relationships between various carbon variables (SOC vs. SIC, δ^{13}C-SIC vs. SIC, δ^{13}C-SIC vs. δ^{13}C-SOC, SIC vs. silt particle, SIC vs. clay particle).

RESULTS

Bulk Density, Soil Particle Content and pH in Shifting Sand Land and Forestlands

Afforestation was found to cause a variation in bulk density and fine particles at 0–40 cm soil layer (**Table 3**). Within this depth, the bulk densities in P-20 land and P-30 land were significantly lower than in SL, but there was no significant difference between

TABLE 2 | Two-way ANOVA for soil carbon content, δ^{13}C-SIC, and δ^{13}C-SOC in for treatments and soil layers.

Soil carbon	Treatment		Layer		Treatment × Layer	
	F	P	F	P	F	P
SOC	935.7	<0.001	39.78	<0.001	7.1	<0.001
SIC	156.86	<0.001	2.27	0.062	1.82	0.046
δ^{13}C-SIC	217.19	<0.001	1.81	0.128	0.31	0.986
δ^{13}C-SOC	150.57	<0.001	6.45	<0.001	1.22	0.267

TABLE 3 | Bulk density, particle content and pH of soil in the four sample plots ($n = 13$, mean ± SD).

Soil properties	Soil depth (cm)	SL	P-8	P-20	P-30
Bulk density (g cm^{-3})	0–20	1.58 ± 0.14 a	1.51 ± 0.14 ab	1.45 ± 0.09 bc	1.43 ± 0.13 c
	20–40	1.56 ± 0.11 a	1.54 ± 0.11 ab	1.47 ± 0.15 bc	1.45 ± 0.14 c
	40–60	1.57 ± 0.13 a	1.52 ± 0.10 a	1.51 ± 0.12 a	1.49 ± 0.11 a
	60–80	1.59 ± 0.12 a	1.56 ± 0.13 a	1.55 ± 0.16 a	1.54 ± 0.09 a
	80–100	1.57 ± 0.09 a	1.58 ± 0.12 a	1.53 ± 0.13 a	1.52 ± 0.14 a
Sand (>0.05 mm, %)	0–20	91.3 ± 3.5 a	90.0 ± 3.8 a	89.7 ± 3.9 a	89.2 ± 2.9 a
	20–40	91.7 ± 2.6 a	90.7 ± 2.4 a	90.5 ± 2.8 a	90.9 ± 3.2 a
	40–60	91.9 ± 2.1 a	91.7 ± 3.2 a	91.1 ± 2.1 a	91.5 ± 3.4 a
	60–80	92.2 ± 3.2 a	91.9 ± 2.9 a	91.7 ± 3.6 a	91.6 ± 3.8 a
	80–100	92.6 ± 3.3 a	92.1 ± 2.8 a	92.6 ± 2.7 a	92.7 ± 3.9 a
Silt (0.002–0.05 mm, %)	0–20	4.8 ± 0.3 b	5.3 ± 0.4 a	5.4 ± 0.3 a	5.8 ± 0.3 a
	20–40	4.7 ± 0.2 b	5.1 ± 0.4 ab	5.0 ± 0.3 ab	5.2 ± 0.5 a
	40–60	4.9 ± 0.3 a	4.8 ± 0.3 a	5.1 ± 0.4 a	4.9 ± 0.3 a
	60–80	4.6 ± 0.2 a	4.9 ± 0.5 a	4.8 ± 0.4 a	4.7 ± 0.3 a
	80–100	4.7 ± 0.4 a	4.8 ± 0.2 a	4.6 ± 0.3 a	4.9 ± 0.4 a
Clay (<0.002 mm, %)	0–20	3.9 ± 0.4 b	4.7 ± 0.3 a	4.9 ± 0.2 a	5.0 ± 0.4 a
	20–40	3.6 ± 0.5 b	4.2 ± 0.4 ab	4.5 ± 0.5 a	3.9 ± 0.3 ab
	40–60	3.2 ± 0.6 a	3.5 ± 0.3 a	3.8 ± 0.5 a	3.6 ± 0.5 a
	60–80	3.2 ± 0.4 a	3.2 ± 0.3 a	3.5 ± 0.3 a	3.7 ± 0.4 a
	80–100	2.7 ± 0.5 a	3.1 ± 0.4 a	2.8 ± 0.4 a	2.4 ± 0.3 a
pH	0–20	8.9 ± 0.3 a	8.6 ± 0.4 ab	8.2 ± 0.3 b	8.1 ± 0.2 b
	20–40	9.0 ± 0.3 a	8.5 ± 0.3 b	8.2 ± 0.2 bc	8.0 ± 0.3 c
	40–60	8.8 ± 0.2 a	8.3 ± 0.4 ab	8.1 ± 0.1 ab	7.9 ± 0.2 b
	60–80	8.7 ± 0.1 a	8.4 ± 0.2 ab	8.2 ± 0.3 b	8.2 ± 0.1 b
	80–100	8.9 ± 0.2 a	8.5 ± 0.3 ab	8.3 ± 0.4 b	8.1 ± 0.3 b

Within each depth, different lowercase letters denote significant differences among the treatments ($P < 0.05$).

P-8 land and SL. The silt and clay particle contents at 0–20 cm in the three forestlands were significantly higher than in SL. At the depth of 20–40 cm, the silt particle content in P-30 land was significantly greater than that in SL, but there was no significant difference between P-8 land and SL or between P-20 land and SL. The clay particle content in P-20 land was remarkably greater than in SL, but there was no significant difference between P-8 land and SL or between P-30 land and SL. Within the 40–100 cm depth layer, no differences in bulk density or fine particles were observed between the four sample plots (**Table 3**). Additionally, soil pH at 0–100 cm in P-20 land and P-30 land was considerably lower than that in SL, but there was no significant difference between P-8 land and SL within the 60–100 cm depth layer (**Table 3**).

SIC in Shifting Sand Land and Forestlands

Soil inorganic carbon content was enhanced by afforestation. Within the 0–100 cm depth, the SIC content in each 20 cm depth interval in P-8, P-20, and P-30 lands was significantly higher than in SL (**Table 4**). Among the three forestlands, the SIC content increased with plantation age. Within the 0–40 cm layer, the SIC content in P-30 land was considerably higher than in P-20 land, but there was no significant difference between P-20 land and P-8 land. Within the 40–100 cm layer, the SIC content in P-30 land was significantly greater than that in P-20 land, which in turn was greater than that in P-8 land. Afforestation also elevated SIC stocks. The SIC stock at 0–100 cm in SL was 34.2 Mg ha^{-1}, which increased to 42.5, 49.2, and 68.3 Mg ha^{-1} in P-8, P-20 and

TABLE 4 | Soil carbon contents in the four sample plots (g kg^{-1}; mean ± standard deviation; $n = 13$).

Soil carbon	Soil depth (cm)	SL	P-8	P-20	P-30
SIC	0–20	2.18 ± 0.22 Ac	2.77 ± 0.61 Ab	3.17 ± 0.38 Ab	5.24 ± 1.16 Aa
	20–40	2.16 ± 0.19 Ac	2.97 ± 0.27 Ab	3.25 ± 0.58 Ab	4.66 ± 0.77 ABa
	40–60	2.17 ± 0.18 Ad	2.64 ± 0.50 Ac	3.55 ± 0.70 Ab	4.59 ± 0.92 ABa
	60–80	2.19 ± 0.19 Ad	2.67 ± 0.57 Ac	3.21 ± 0.70 Ab	4.55 ± 0.86 ABa
	80–100	2.17 ± 0.20 Ad	2.74 ± 0.33 Ac	3.09 ± 0.58 Ab	3.84 ± 0.58 Ba
SOC	0–20	0.40 ± 0.03 Ad	2.00 ± 0.20 Ac	4.53 ± 0.86 Ab	6.11 ± 1.03 Aa
	20–40	0.38 ± 0.02 Ad	1.54 ± 0.21 Bc	3.88 ± 0.79 Bb	4.98 ± 0.80 Ba
	40–60	0.42 ± 0.04 Ad	1.34 ± 0.21 BCc	3.40 ± 0.59 BCb	4.69 ± 0.77 Ba
	60–80	0.38 ± 0.03 Ad	1.20 ± 0.23 CDc	3.21 ± 0.38 BCb	4.03 ± 0.40 Ca
	80–100	0.40 ± 0.03 Ad	1.03 ± 0.18 Dc	3.00 ± 0.45 Cb	3.43 ± 0.37 Ca

Within each treatment, different uppercase letters denote significant differences among the depths ($P < 0.05$); within each depth, different lowercase letters denote significant differences among the treatments ($P < 0.05$).

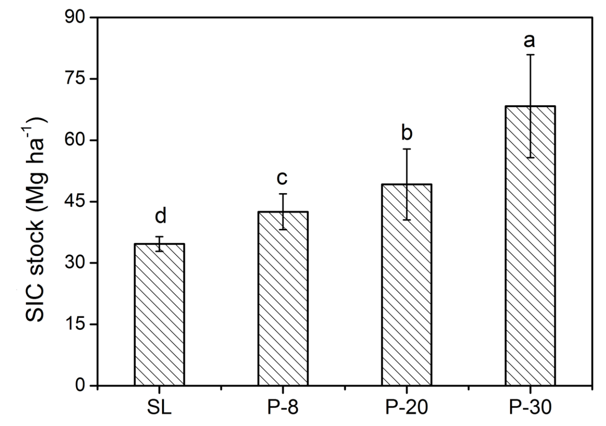

FIGURE 2 | Soil inorganic carbon (SIC) stocks within 0–100 cm in shifting sand land (SL), 8-year-old poplar (P-8) land, 20-year-old poplar (P-20) land, and 30-year-old poplar (P-30) land (mean ± SD; *n* = 13). Different lowercase letters denote significant differences among the treatments (*P* < 0.05).

P-30 lands, respectively (**Figure 2**). The SIC contents in SL, P-8 land and P-20 land were almost evenly distributed among the five 20 cm soil intervals from 0 to 100 cm (**Table 4**). The SIC content in P-30 land at 0–20 cm was significantly higher than at 80–100 cm; however, no differences were observed among the 0–80 cm layers or among the 20–100 cm layers. In addition, the SOC content in the three forestlands was significantly higher in each soil layer than at the same depth in SL (**Table 4**).

δ^{13}C-SIC and δ^{13}C-SOC in Shifting Sand Land and Forestlands

In P-8, P-20, P-30 and SL lands, the δ^{13}C-SIC values showed little vertical variation throughout the 0–100 cm soil layers (**Table 5**). Among the four sample plots, the δ^{13}C-SIC values in SL land were the highest in all five soil layers, and δ^{13}C-SIC value decreased with plantation age after afforestation. At 0–80 cm, the δ^{13}C-SIC values in P-30 land were significantly lower than those in P-20 land, which in turn were lower than those in P-8 land. At 80–100 cm, δ^{13}C-SIC value in P-30 land was also the lowest, but no difference was observed at this layer between P-20 land and P-8 land. The δ^{13}C-SOC values within the 0–60 cm depth showed a gradual decrease with plantation age after afforestation. At 60–100 cm, the δ^{13}C-SOC values were not significantly different between SL land and P-8 land, but these values in the both plots were dramatically higher than those in P-20 land and P-30 land (**Table 5**).

Figure 3 shows a strong correlation between SIC content and δ^{13}C-SIC. Using all 260 samples, the relationship between δ^{13}C-SIC content and SIC was shown to fit a linear model, and δ^{13}C-SIC was observed to explain more than 70% of the variation in SIC ($R^2 = 0.71$, $P < 0.01$). Our data also showed that the variations in SIC and δ^{13}C-SIC were related to SOC and δ^{13}C-SOC. There was a positive linear relationship ($R^2 = 0.52$, $P < 0.01$) between SOC and SIC content for all soil samples (**Figure 4**). The entire dataset (260 samples) exhibited a positive correlation between δ^{13}C-SOC and δ^{13}C-SIC ($R^2 = 0.63$, $P < 0.01$, **Figure 5**). Additionally, there was no obvious correlation between silt particle content and SIC content (**Figure 6A**) or between clay particle content and SIC content (**Figure 6B**).

DISCUSSION

SIC Sequestration Following Afforestation and the Contribution of Soil Fine Particles to SIC Sequestration

Our results showed that the SIC stock at depth of 0–100 cm in SL was 34.2 Mg ha^{-1} and that it gradually increased along the chronosequence of afforestation (**Figure 2**). The results were consistent with those reported by Su et al. (2010) and Li Y.Q. et al. (2013), who also observed that SIC increased markedly with plantation age after afforestation on SL. However, our findings were in disagreement with some earlier reports in semiarid regions. In the Columbia Plateau, Oregon, United States, after 10 years, poplar plantations in a desert reduced the SIC concentration from 2.6 to 1.2 g kg^{-1} in the surface layer (Sartori et al., 2007). In the Chinese Loess Plateau, Wang et al.

TABLE 5 | δ^{13}C-SIC and δ^{13}C-SOC in the four sample plots (‰; mean ± standard deviation; $n = 13$).

	Soil depth (cm)	SL	P-8	P-20	P-30
δ^{13}C-SIC	0–20	−4.08 ± 0.27 Aa	−4.72 ± 0.55 Ab	−5.59 ± 0.65 Ac	−6.72 ± 0.69 Ad
	20–40	−4.04 ± 0.23 Aa	−5.08 ± 0.63 Aa	−5.90 ± 0.47 Ab	−6.41 ± 0.68 Ac
	40–60	−4.00 ± 0.26 Aa	−4.71 ± 0.70 Ab	−5.69 ± 0.51 Ac	−6.55 ± 0.66 Ad
	60–80	−4.05 ± 0.23 Aa	−4.95 ± 0.77 Ab	−5.87 ± 0.63 Ac	−6.69 ± 0.76 Ad
	80–100	−4.06 ± 0.23 Aa	−5.17 ± 0.63 Ab	−5.74 ± 0.62 Ab	−6.58 ± 0.70 Ac
δ^{13}C-SOC	0–20	−18.68 ± 1.54 Aa	−23.36 ± 2.00 Bb	−25.44 ± 1.90 Bb	−27.60 ± 2.20 Bc
	20–40	−18.82 ± 1.66 Aa	−21.46 ± 2.31 ABb	−24.41 ± 1.77 ABc	−26.75 ± 2.28 ABd
	40–60	−19.08 ± 1.89 Aa	−21.60 ± 1.98 ABb	−24.39 ± 1.52 ABc	−25.49 ± 1.47 ABc
	60–80	−19.37 ± 2.06 Aa	−20.84 ± 2.13 Aa	−23.83 ± 1.78 ABb	−25.50 ± 2.30 ABb
	80–100	−18.71 ± 1.81 Aa	−20.34 ± 2.43 Aa	−23.34 ± 1.45 Ab	−24.81 ± 2.42 Ab

Within each treatment, different uppercase letters denote significant differences among the depths ($P < 0.05$); within each depth, different lowercase letters denote significant differences among the treatments ($P < 0.05$).

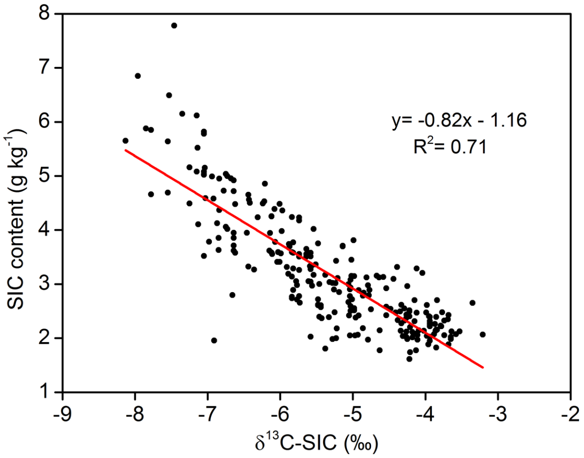

FIGURE 3 | Relationship between soil inorganic δ^{13}C value (δ^{13}C-SIC) and inorganic carbon (SIC) contents (using all 260 samples within 0–100 cm depth from four sample plots). Significance of the linear regression was considered as $P < 0.01$.

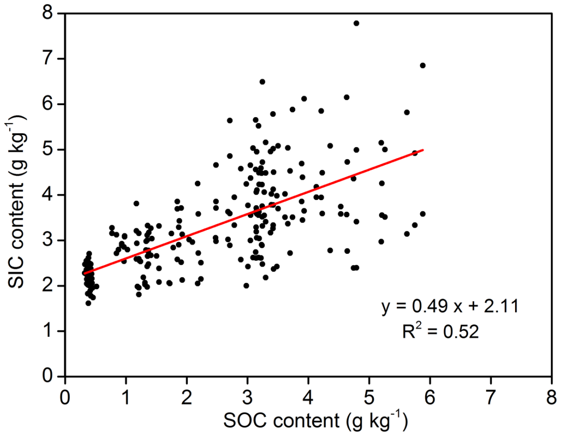

FIGURE 4 | Relationship between soil organic carbon (SOC) and inorganic carbon (SIC) contents (using all 260 samples within 0–100 cm depth from four sample plots). Significance of the linear regression was considered as $P < 0.01$.

(2016) reported that the SIC storage at depth of 0–100 cm in the farmland was significantly lower than that in the restored artificial forestland, with a difference of 16.8 Mg ha^{-1}. The SIC reduction in these inconsistent findings was mainly caused by irrigation or surface runoff, which can remove mass containing dissolved inorganic carbon. In the present study, similar processes would not be applicable because there was no irrigation or heavy rainfall. Therefore, our findings indicate that afforestation on shifting SL has a high potential to sequester SIC in degraded semiarid regions.

One theory posits that soil fine particles may play an important role in SIC sequestration following afforestation (Li et al., 2012). Plant canopies can intercept and deposit fine particles from the wind-sand flow after afforestation. This sediment contains rich carbonate sources, such as calcite, and causes a rapid SIC accumulation in surface soil (0–20 cm) (Wang et al., 2006). However, we found that this theory could not provide a complete explanation for SIC accumulation. Afforestation on SL not only elevates SIC stock in the surface soil layer, but also increases SIC levels in the deeper layers (**Table 4**; Li Y.Q. et al., 2013). Nevertheless, afforestation enhanced fine particles only at a depth of 0–40 cm, but not in the 40–100 cm depths (**Table 3**). In the deep layers (>40 cm), soil fine particles stack at an exceptionally slow rate and contribute little to SIC sequestration (Li et al., 2007). Moreover, we detected no correlation between fine particles and SIC content in the present study (**Figure 6**), further suggesting that the contribution of soil fine particles by the canopy to SIC sequestration is limited for the 0–100 cm soil layer. This phenomenon indicates that SIC sequestration is not exclusively derived from fine particle deposition and that other SIC accumulation processes may be occurring after afforestation.

Effects of Afforestation on Stable Carbon Isotopes and Implications for Revealing the Mechanism of SIC Sequestration

We found that δ^{13}C-SIC decreased with plantation age in forestlands (**Table 5**). Wang J.P. et al. (2015) found that the

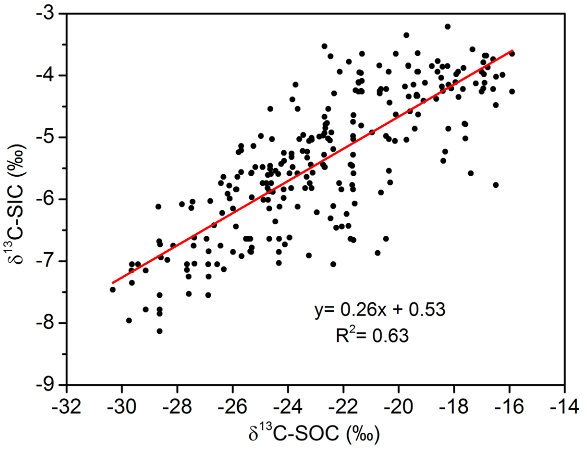

FIGURE 5 | Relationships of soil inorganic δ^{13}C value (δ^{13}C-SIC) with soil organic δ^{13}C value (δ^{13}C-SOC) (using all 260 samples within 0–100 cm depth from four sample plots). Significance of the linear regression was considered as $P < 0.01$.

δ^{13}C-SIC for desert soil was significantly higher than that for shrubland soil on the northeastern edge of the Taklamakan Desert, China. Liu et al. (2014) also pointed out that the δ^{13}C value of soil carbonate along a chronosequence decreased gradually with vegetation restoration. SIC is composed of the LIC and PIC, which have distinct δ^{13}C-SIC values. The changes in δ^{13}C-SIC following vegetation rehabilitation can be used to explain the reason for SIC variation (Stevenson et al., 2005). There is sufficient evidence that the decrease in δ^{13}C-SIC indicates PIC formation when land use patterns change (Jin et al., 2014; Liu et al., 2014; Wang et al., 2014, 2016; Wang J.P. et al., 2015; Bughio et al., 2016). Accordingly, the decrease in δ^{13}C-SIC with plantation age in our study indicates that afforestation induced abundant PIC formation. Furthermore, a strong negative linear relationship between δ^{13}C-SIC and SIC content in our study (**Figure 3**), which was also observed by Wang X.J. et al. (2015) in the northwest China, suggests that a decreasing δ^{13}C-SIC is associated with SIC sequestration following afforestation. Specifically, PIC formation is accompanied by SIC sequestration, as the decrease in δ^{13}C-SIC is indicative of the formation of PIC. Therefore, the carbon isotope data in this study indicate that SIC sequestration is probably caused by PIC formation after afforestation on SL. Additionally, an estimation of the amount of PIC would be very important to better understanding the contribution of PIC to SIC sequestration. Based on the precise δ^{13}C-SIC, δ^{13}C-PIC and δ^{13}C-LIC values and empirical formulas, Wang et al. (2014) successfully estimated the accumulation rate of PIC under fertilization for loess soil. This method can ostensibly be used to calculate the amount of PIC in the forestlands in our study. However, an accurate δ^{13}C-LIC value in the desert cannot be measured with the current technology, so we cannot supply values for the PIC stocks in this study. The δ^{13}C-LIC of desert soil should be precisely identified in future studies because it is crucial for quantifying PIC stock.

Effect of SOC Accumulation on PIC Formation

In this study, afforestation simultaneously enhanced SIC and SOC contents (**Figure 2**), and SIC content was positively correlated with SOC content (**Figure 4**). Similar relationships have also been identified in other arid and semiarid regions in China (Zhang N. et al., 2010; Wang X.J. et al., 2015; Guo et al., 2016). These results suggest that the increase of SIC following

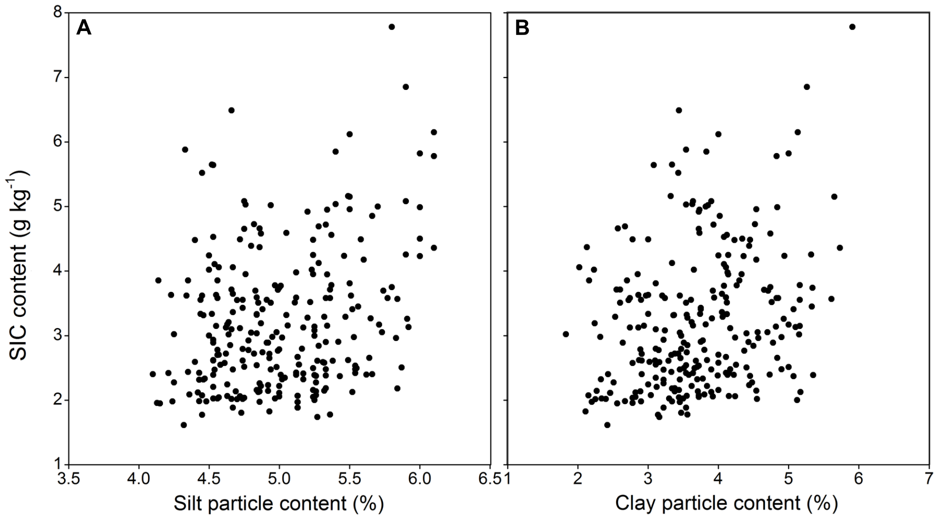

FIGURE 6 | Relationships of soil inorganic δ^{13}C value (δ^{13}C-SIC) with silt particle content and clay particle content (using all 260 samples within 0–100 cm depth from four sample plots). **(A)** Relationship between δ^{13}C-SIC and silt particle content; **(B)** relationship between δ^{13}C-SIC and clay particle content. Significance of the linear regression was considered as $P < 0.01$.

afforestation may be related to SOC accumulation. Furthermore, our results showed that there was a decrease in both δ^{13}C-SIC and δ^{13}C-SOC with plantation age. δ^{13}C-SIC was strongly positively correlated with δ^{13}C-SOC (**Figure 5**), a finding that is consistent with the observations of Landi et al. (2003). In other words, the decrease in δ^{13}C-SIC was accompanied by a decrease in δ^{13}C-SOC. The decrease in δ^{13}C-SIC indicates PIC formation, and SOC accumulation invariably leads to a decrease in δ^{13}C-SOC due to plant litter input (Trolier et al., 1996; Jin et al., 2014). These results further imply that the PIC formation following afforestation is correlated with SOC accumulation. Soil organic matter affected PIC formation by regulating soil CO_2 concentration and the precipitation of carbonate in the alkaline environment (Monger et al., 2015). PIC accumulation involves two main reactions:

$$2CO_2 + 2H_2O \leftrightarrow 2HCO_3^- + 2H^+ \qquad (3)$$

$$Ca^{2+} + 2HCO_3^- \leftrightarrow CaCO_3 + H_2O + CO_2 \qquad (4)$$

A mass of CO_2 is released into the soil following shrub and tree plantation in deserts, mainly due to the decomposition of the increased amount of organic matter (Zhang Z.S. et al., 2013). In general, an increase in soil CO_2 concentration would lead to the production of HCO_3^-. The accumulated HCO_3^- can drive reaction (4) to the right, resulting in the precipitation of carbonate (Wang X.J. et al., 2015; Zamanian et al., 2016). When 2 mole of CO_2 is consumed, 1 mole of $CaCO_3$ is generated. At our study site, the soil has a pH greater than 8 (**Table 3**) and is rich in available Ca^{2+} and Mg^{2+} (**Table 1**). The decomposition of the increased SOC in forestlands would dramatically elevate the soil CO_2 concentration and facilitate the occurrence of reaction (3). The alkaline environmental conditions could neutralize the H^+ from reaction (3), which may be the reason for the decline in pH in forestlands (**Table 3**). These conditions also continuously promote the formation of HCO_3^-. The newly generated HCO_3^- combined with available cations may cause PIC accumulation following afforestation (Meyer et al., 2014; Monger et al., 2015). In addition to the CO_2 emitted via decomposition of the increased SOC, soil CO_2 respired by the roots of poplar trees (autotrophic respiration) would affect the formation of PIC. The effects of autotrophic respiration on PIC formation in plantation lands need to be studied in future. Additionally, a long-term study by observing SIC, SOC, soil carbon isotopes, soil CO_2 concentration and available cations in the same forestland is required, which could more directly and precisely characterize the mechanisms of SIC variation along a chronosequence of afforestation.

CONCLUSION

Our data demonstrate that afforestation on shifting SL has a high potential to sequester SIC in degraded semiarid regions. Afforestation elevated soil fine particles only at 0–40 cm, and there was no correlation between SIC content and soil fine particles, suggesting that the contribution of soil fine particle deposition to SIC accumulation is limited. The decrease in δ^{13}C-SIC along a chronosequence of forestland and the relationship between δ^{13}C-SIC and SIC content both indicate that SIC sequestration following afforestation is probably caused by PIC formation. The positive correlations between SIC content and SOC content and between δ^{13}C-SIC and δ^{13}C-SOC imply that the newly formed PIC may be closely related to SOC accumulation. Our findings suggest that SIC plays an important role in the carbon cycle in semiarid areas and that by overlooking SIC, we may substantially underestimate carbon sequestration capacities following vegetation rehabilitation. Our stable carbon isotope data will help to form an understanding of the mechanisms of SIC formation and transformation in arid and semiarid areas.

AUTHOR CONTRIBUTIONS

JL designed the experiment; YG, JT, and YP carried out the field work; YG and JL analyzed the data; YG wrote the manuscript; and JL assisted with revising the draft manuscript.

FUNDING

This research was supported by the National Natural Science Foundation of China (No. 31500585), Fundamental Research Fund for the Central Universities (No. Z109021619) and Natural Science Foundation of Shaanxi Province (No. 2016JQ3021).

ACKNOWLEDGMENT

The authors thank Zhen Liu, Yuxuan Bai, Shijun Liu for their assistance in the field and laboratory.

REFERENCES

Bughio, M. A., Wang, P. L., Meng, F. Q., Qing, C., Kuzyakov, Y., Wang, X. J., et al. (2016). Neoformation of pedogenic carbonates by irrigation and fertilization and their contribution to carbon sequestration in soil. *Geoderma* 262, 12–19. doi: 10.1016/j.geoderma.2015.08.003

Cerling, T. E., Quade, J., Wang, Y., and Bowman, J. R. (1989). Carbon isotopes in soils and palaeosols as ecology and palaeoecology indicators. *Nature* 341, 138–139. doi: 10.1038/341138a0

Chang, R. Y., Fu, B. J., Liu, G. B., Wang, S., and Yao, X. L. (2012). The effects of afforestation on soil organic and inorganic carbon: a case study of the Loess Plateau of China. *Catena* 95, 145–152. doi: 10.1016/j.catena.2012.02.012

Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Commun. Mass Spectrom.* 25, 2538–2560. doi: 10.1002/rcm.5129

Delgado-Baquerizo, M., Maestre, F. T., Gallardo, A., Bowker, M. A., Wallenstein, M. D., Quero, J. L., et al. (2013). Decoupling of soil nutrient cycles as a function of aridity in global drylands. *Nature* 502, 672–676. doi: 10.1038/nature12670

Deng, L., Liu, G. B., and Shangguan, Z. P. (2014). Land-use conversion and changing soil carbon stocks in China's "Grain-for-Green" program: a synthesis. *Global Change Biol.* 20, 3544–3556. doi: 10.1111/gcb.12508

Farley, K. A., Kelly, E. F., and Hofstede, R. G. M. (2004). Soil organic carbon and water retention after conversion of grasslands to pine plantations in the Ecuadorian Andes. *Ecosystems* 7, 729–739. doi: 10.1007/s10021-004-0047-5

Gao, G. L., Ding, G. D., Zhao, Y. Y., Wu, B., Zhang, Y. Q., Qin, S. G., et al. (2014). Fractal approach to estimating changes in soil properties following the establishment of *Caragana korshinskii* shelterbelts in Ningxia, NW China. *Ecol. Indic.* 43, 236–243. doi: 10.1016/j.ecolind.2014.03.001

Guo, Y., Wang, X. J., Li, X. L., Wang, J. P., Xu, M. G., and Li, D. W. (2016). Dynamics of soil organic and inorganic carbon in the cropland of upper Yellow River Delta, China. *Sci. Rep.* 6:36105. doi: 10.1038/srep36105

Huang, G., Zhao, X. Y., Li, Y. Q., and Cui, J. Y. (2012). Restoration of shrub communities elevates organic carbon in arid soils of northwestern China. *Soil Biol. Biochem.* 47, 123–132. doi: 10.1016/j.soilbio.2011.12.025

Jackson, R. B., Banner, J. L., Jobbágy, E. G., Pockman, W. T., and Wall, D. H. (2002). Ecosystem carbon loss with woody plant invasion of grasslands. *Nature* 418, 623–626. doi: 10.1038/nature00910

Jin, Z., Dong, Y. S., Wang, Y. Q., Wei, X. R., Wang, Y. F., Cui, B. L., et al. (2014). Natural vegetation restoration is more beneficial to soil surface organic and inorganic carbon sequestration than tree plantation on the Loess Plateau of China. *Sci. Total Environ.* 48, 615–623. doi: 10.1016/j.scitotenv.2014.03.105

Jobbágy, E. G., and Jackson, R. B. (2003). Patterns and mechanisms of soil acidification in the conversion of grasslands to forests. *Biogeochemistry* 64, 205–229. doi: 10.1023/A:1024985629259

Lal, R. (2004). Soil carbon sequestration impacts on global climate change and food security. *Science* 304, 1623–1627. doi: 10.1126/science.1097396

Lal, R. (2009). Sequestering carbon in soils of arid ecosystems. *Land Degrad. Dev.* 20, 441–454. doi: 10.1002/ldr.934

Lal, R. (2010). Managing soils and ecosystems for mitigating anthropogenic carbon emissions and advancing global food security. *Bioscience* 60, 708–721. doi: 10.1525/bio.2010.60.9.8

Landi, A., Mermut, A. R., and Anderson, D. W. (2003). Origin and rate of pedogenic carbonate accumulation in Saskatchewan soils, Canada. *Geoderma* 117, 143–156. doi: 10.1016/S0016-7061(03)00161-7

Li, C. F., Zhang, C., Luo, G. P., Chen, X., Maisupova, B., Madaminov, A. A., et al. (2015). Carbon stock and its responses to climate change in Central Asia. *Glob. Change Biol.* 21, 1951–1967. doi: 10.1111/gcb.12846

Li, G. J., Chen, J., and Chen, Y. (2013). Primary and secondary carbonate in Chinese loess discriminated by trace element composition. *Geochim. Cosmochim. Acta* 103, 26–35. doi: 10.1016/j.gca.2012.10.049

Li, X. J., Li, X. R., Wang, X. P., and Yang, H. T. (2016). Changes in soil organic carbon fractions after afforestation with xerophytic shrubs in the Tengger Desert, northern China. *Eur. J. Soil Sci.* 67, 184–195. doi: 10.1111/ejss.12315

Li, X. R., Kong, D. S., Tan, H. J., and Wang, X. P. (2007). Changes in soil and vegetation following stabilisation of dunes in the southeastern fringe of the Tengger Desert, China. *Plant Soil* 300, 221–231. doi: 10.1007/s11104-007-9407-1

Li, Y. Q., Awada, T., Zhou, X. H., Shang, W., Chen, Y. P., Zuo, X. A., et al. (2012). Mongolian pine plantations enhance soil physico-chemical properties and carbon and nitrogen capacities in semi-arid degraded sandy land in China. *Appl. Soil Ecol.* 56, 1–9. doi: 10.1016/j.apsoil.2012.01.007

Li, Y. Q., Brandle, J., Awada, T., Chen, Y. P., Han, J. J., Zhang, F. X., et al. (2013). Accumulation of carbon and nitrogen in the plant–soil system after afforestation of active sand dunes in China's Horqin Sandy Land. *Agric. Ecosyst. Environ.* 177, 75–84. doi: 10.1016/j.agee.2013.06.007

Liu, R. T., Zhao, H. L., and Zhao, X. Y. (2013). Changes in soil macrofaunal community composition under selective afforestation in shifting sand lands in Horqin of Inner Mongolia, northern China. *Ecol. Res.* 28, 1–8. doi: 10.1007/s11284-012-0982-0

Liu, W. G., Wei, J., Cheng, J. M., and Li, W. J. (2014). Profile distribution of soil inorganic carbon along a chronosequence of grassland restoration on a 22-year scale in the Chinese Loess Plateau. *Catena* 121, 321–329. doi: 10.1016/j.catena.2014.05.019

Meyer, N. A., Breecker, D. O., Young, M. H., and Litvak, M. E. (2014). Simulating the effect of vegetation in formation of pedogenic carbonate. *Soil Sci. Soc. Am. J.* 78, 914–924. doi: 10.2136/sssaj2013.08.0326

Mi, N., Wang, S. Q., Liu, J. Y., Yu, G. R., Zhang, W. J., and Jobbágy, E. (2008). Soil inorganic carbon storage pattern in China. *Glob. Chang. Biol.* 14, 2380–2387. doi: 10.1111/j.1365-2486.2008.01642.x

Mielnick, P., Dugas, W. A., Mitchell, K., and Havstad, K. (2005). Long-term measurements of CO2 flux and evapotranspiration in a Chihuahuan desert grassland. *J. Arid. Environ.* 60, 423–436. doi: 10.1016/j.jaridenv.2004.06.001

Monger, H. C., Kraimer, R. A., Khresat, S., Cole, D. R., Wang, X. J., and Wang, J. P. (2015). Sequestration of inorganic carbon in soil and groundwater. *Geology* 43, 375–378. doi: 10.1130/G36449.1

Qiu, L., Wei, X., Gao, J., and Zhang, X. (2015). Dynamics of soil aggregate-associated organic carbon along an afforestation chronosequence. *Plant Soil* 391, 237–251. doi: 10.1007/s11104-015-2415-7

Rao, Z. G., Zhu, Z. Y., Chen, F. H., and Zhang, J. W. (2006). Does δ13Ccarb of the Chinese loess indicate past C3/C4 abundance? A review of research on stable carbon isotopes of the Chinese loess. *Quat. Sci. Rev.* 25, 2251–2257. doi: 10.1016/j.quascirev.2006.03.013

Reynolds, J. F., Smith, D. M. S., Lambin, E. F., Turner, B. L. II, Mortimore, M., Batterbury, S. P. J., et al. (2007). Global desertification: building a science for dryland development. *Science* 316, 847–851. doi: 10.1126/science.1131634

Sartori, F., Lal, R., Ebinger, M. H., and Eaton, J. A. (2007). Changes in soil carbon and nutrient pools along a chronosequence of poplar plantations in the Columbia Plateau, Oregon, USA. *Agric. Ecosyst. Environ.* 122, 325–339. doi: 10.1016/j.agee.2007.01.026

Schlesinger, W. H. (1982). Carbon storage in the Caliche of arid soils: a case study from Arizona. *Soil Sci.* 133, 247–255. doi: 10.1097/00010694-198204000-00008

Stevenson, B. A., Kelly, E. F., McDonald, E. V., and Busacca, A. J. (2005). The stable carbon isotope composition of soil organic carbon and pedogenic carbonates along a bioclimatic gradient in the Palouse region, Washington State, USA. *Geoderma* 124, 37–47. doi: 10.1016/j.geoderma.2004.03.006

Su, Y. Z., Wang, X. F., Yang, R., and Lee, J. (2010). Effects of sandy desertified land rehabilitation on soil carbon sequestration and aggregation in an arid region in China. *J. Environ. Manage.* 91, 2109–2116. doi: 10.1016/j.jenvman.2009.12.014

Su, Y. Z., and Zhao, H. L. (2003). Soil properties and plant species in an age sequence of *Caragana microphylla* plantations in the Horqin Sandy Land, north China. *Ecol. Eng.* 20, 223–235. doi: 10.1016/S0925-8574(03)00042-9

Tan, W. F., Zhang, R., Cao, H., Huang, C. Q., Yang, Q. K., Wang, M. K., et al. (2014). Soil inorganic carbon stock under different soil types and land uses on the Loess Plateau region of China. *Catena* 121, 22–30. doi: 10.1016/j.catena.2014.04.014

Trolier, M., White, J. W. C., Tans, P. P., Masarie, K. A., and Gemery, P. A. (1996). Monitoring the isotopic composition of atmospheric CO2: measurements from the NOAA global air sampling network. *J. Geophys. Res.* 101, 25897–25916. doi: 10.1029/96JD02363

Walkley, A., and Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* 37, 29–38. doi: 10.1097/00010694-193401000-00003

Wang, J. P., Monger, C., Wang, X. J., Serena, M., and Leinauer, B. (2016). Carbon sequestration in response to grassland–shrubland–turfgrass conversions and a test for carbonate biomineralization in desert soils, New Mexico, USA. *Soil Sci. Soc. Am. J.* 80, 1591–1603. doi: 10.2136/sssaj2016.03.0061

Wang, J. P., Wang, X. J., Zhang, J., and Zhao, C. Y. (2015). Soil organic and inorganic carbon and stable carbon isotopes in the Yanqi Basin of northwestern China. *Eur. J. Soil Sci.* 66, 95–103. doi: 10.1111/ejss.12188

Wang, X. J., Wang, J. P., Xu, M. G., Zhang, W. J., Fan, T. L., and Zhang, J. (2015). Carbon accumulation in arid croplands of northwest China: pedogenic carbonate exceeding organic carbon. *Sci. Rep.* 5:11439. doi: 10.1038/srep11439

Wang, X. J., Wang, J. P., and Zhang, J. (2012). Comparisons of three methods for organic and inorganic carbon in calcareous soils of Northwestern China. *PLoS ONE* 7:e44334. doi: 10.1371/journal.pone.0044334

Wang, X. J., Xu, M. G., Wang, J. P., Zhang, W. J., Yang, X. Y., Huang, S. M., et al. (2014). Fertilization enhancing carbon sequestration as carbonate in arid cropland: assessments of long-term experiments in northern China. *Plant Soil* 380, 89–100. doi: 10.1007/s11104-014-2077-x

Wang, X. P., Li, X. R., Xiao, H. L., and Pan, Y. X. (2006). Evolutionary characteristics of the artificially revegetated shrub ecosystem in the Tengger Desert, northern China. *Ecol. Res.* 21, 415–424. doi: 10.1007/s11284-005-0135-9

Wang, Y. G., Li, Y., Ye, X. H., Chu, Y., and Wang, X. P. (2010). Profile storage of organic/inorganic carbon in soil: from forest to desert. *Sci. Total Environ.* 408, 1925–1931. doi: 10.1016/j.scitotenv.2010.01.015

Zamanian, K., Pustovoytov, K., and Kuzyakov, Y. (2016). Pedogenic carbonates: Forms and formation processes. *Earth Sci. Rev.* 157, 1–17. doi: 10.1016/j.earscirev.2016.03.003

Zhang, F., Wang, X. J., Guo, T. W., Zhang, P. L., and Wang, J. P. (2015). Soil organic and inorganic carbon in the loess profiles of Lanzhou area: implications of deep soils. *Catena* 126, 68–74. doi: 10.1016/j.catena.2014.10.031

Zhang, K., Dang, H., Tan, S., Cheng, X., and Zhang, Q. (2010). Change in soil organic carbon following the 'Grain-For-Green' programme in China. *Land Degrad. Dev.* 21, 13–23. doi: 10.1002/ldr.954

Zhang, N., He, X. D., Gao, Y. B., Li, Y. H., Wang, H. T., Ma, D., et al. (2010). Pedogenic carbonate and soil dehydrogenase activity in response to soil organic matter in *Artemisia ordosica* community. *Pedosphere* 20, 229–235. doi: 10.1016/S1002-0160(10)60010-0

Zhang, Y., Cao, C. Y., Han, X. S., and Jiang, S. Y. (2013). Soil nutrient and microbiological property recoveries via native shrub and semi-shrub plantations on moving sand dunes in Northeast China. *Ecol. Eng.* 53, 1–5. doi: 10.1016/j.ecoleng.2013.01.012

Zhang, Z. S., Li, X. R., Nowak, R. S., Wu, P., Gao, Y. H., Zhao, Y., et al. (2013). Effect of sand-stabilizing shrubs on soil respiration in a temperate desert. *Plant Soil* 367, 449–463. doi: 10.1007/s11104-012-1465-3

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Plant Science

ORIGINAL RESEARCH
published: 04 September 2017
doi: 10.3389/fpls.2017.01523

Soil Biological Activity Contributing to Phosphorus Availability in Vertisols under Long-Term Organic and Conventional Agricultural Management

Nisar A. Bhat[1], Amritbir Riar[2], Aketi Ramesh[3], Sanjeeda Iqbal[1], Mahaveer P. Sharma[3], Sanjay K. Sharma[4] and Gurbir S. Bhullar[2]*

[1] Government Holkar Science College, Devi Ahilya Vishwavidyalaya, Indore, India, [2] Department of International Cooperation, Research Institute of Organic Agriculture (FiBL), Frick, Switzerland, [3] ICAR-Indian Institute of Soybean Research, Indore, India, [4] Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya Agriculture College, Indore, India

Mobilization of unavailable phosphorus (P) to plant available P is a prerequisite to sustain crop productivity. Although most of the agricultural soils have sufficient amounts of phosphorus, low availability of native soil P remains a key limiting factor to increasing crop productivity. Solubilization and mineralization of applied and native P to plant available form is mediated through a number of biological and biochemical processes that are strongly influenced by soil carbon/organic matter, besides other biotic and abiotic factors. Soils rich in organic matter are expected to have higher P availability potentially due to higher biological activity. In conventional agricultural systems mineral fertilizers are used to supply P for plant growth, whereas organic systems largely rely on inputs of organic origin. The soils under organic management are supposed to be biologically more active and thus possess a higher capability to mobilize native or applied P. In this study we compared biological activity in soil of a long-term farming systems comparison field trial in vertisols under a subtropical (semi-arid) environment. Soil samples were collected from plots under 7 years of organic and conventional management at five different time points in soybean (*Glycine max*) -wheat (*Triticum aestivum*) crop sequence including the crop growth stages of reproductive significance. Upon analysis of various soil biological properties such as dehydrogenase, β-glucosidase, acid and alkaline phosphatase activities, microbial respiration, substrate induced respiration, soil microbial biomass carbon, organically managed soils were found to be biologically more active particularly at R2 stage in soybean and panicle initiation stage in wheat. We also determined the synergies between these biological parameters by using the methodology of principle component analysis. At all sampling points, P availability in organic and conventional systems was comparable. Our findings clearly indicate that owing to higher biological activity, organic systems possess equal capabilities of supplying P for crop growth as are conventional systems with inputs of mineral P fertilizers.

Keywords: biological properties, phosphorus mobilization, soil enzymes, soybean–wheat system, available P

Edited by:
Ludmilla Aristilde,
Cornell University, United States

Reviewed by:
Stephen J. Ventura,
University of Wisconsin-Madison,
United States
Paul John Hunter,
University of Warwick,
United Kingdom

***Correspondence:**
Gurbir S. Bhullar
gurbir.bhullar@fibl.org

Specialty section:
This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Plant Science

***Received:** 24 May 2017*
***Accepted:** 21 August 2017*
***Published:** 04 September 2017*

Citation:
Bhat NA, Riar A, Ramesh A, Iqbal S, Sharma MP, Sharma SK and Bhullar GS (2017) Soil Biological Activity Contributing to Phosphorus Availability in Vertisols under Long-Term Organic and Conventional Agricultural Management. Front. Plant Sci. 8:1523. doi: 10.3389/fpls.2017.01523

INTRODUCTION

Low availability of native soil phosphorus for plant growth acts as a limiting factor to realize increased crop productivity (Lynch and Brown, 2008; Khan and Joergensen, 2009; Malik et al., 2012; Johnston et al., 2014). It is well known that most of the soils contain appreciable amounts of total P, yet soil solution P concentrations are ironically low and thereby an impediment for sufficient plant P assimilation (Hinsinger, 2001). As P is subjected to precipitation reactions and sorption reactions on soil colloids, substantial proportions of applied and native soil P are rendered unavailable (Alam and Ladha, 2004; Brady and Weil, 2008; Khan and Joergensen, 2009). Therefore, owing to the very low efficiency of applied P (Syers et al., 2008), large amounts of fertilizer P are required to sufficiently increase soil solution P concentrations for assimilation by crop plants to sustain crop productivity (Zhang et al., 2010; Shen et al., 2011; Bai et al., 2013). Inorganic P fertilizers are, however, costly and are either out of the reach of resource poor farmers in most of the developing countries or need to be heavily subsidized by tax payers' money. Furthermore, with rapidly diminishing accessible natural P resources, relying solely on inorganic P fertilizers is not a sustainable strategy (Cordell et al., 2009). Therefore, it is of high importance that alternate agricultural management strategies are devised that are cost effective, P efficient and sustainable (Harvey et al., 2009; Sánchez, 2010). Apart from the input of mineral P fertilizers, some of the agricultural strategies that can mobilize soil P for plant assimilation include organic matter management (Damodar Reddy et al., 1999; Aulakh et al., 2003; Singh et al., 2007), tillage interventions (Basamba et al., 2006; Shi et al., 2013), microbial inoculation (Ramesh et al., 2011, 2014; Kumar et al., 2014), and crop rotation (Aulakh et al., 2003; Ciampitti et al., 2011).

In nature, phosphorus is known to occur in a number of discrete chemical forms varying in solubility and availability. In agricultural soils, P is found in both inorganic and organic forms, of which organic forms of P are predominant (Turner et al., 2002; Condron et al., 2005; Kong et al., 2009; Richardson et al., 2011). Most of the organic P exists as phytate-P and in lesser amounts as other phosphate esters such as phospholipids (Turner et al., 2007; Richardson et al., 2011). The presence of high phytate-P in soils could be attributed to its low solubility and close affinity toward the solid phase (soil colloids) because of its higher stability (George et al., 2005; Tang et al., 2006). This has been a major impediment to P availability for plant uptake. Availability of P for crop assimilation is net resultant of a number of simultaneously occurring processes, predominantly the mobilization of inorganic P, mineralization of organic P, immobilization of applied P and the rates of P diffusion. These processes are influenced and mediated by several bio-chemical and microbiological activities. Though the roles of most of these biological activities in specific processes are well understood, their synergistic or antagonistic functions and their interactions under particular management environments are still poorly studied.

By improving soil physicochemical and biological properties, organic farming systems are known to play an important role in agricultural ecosystems. They are also advocated for their contribution to nutrient cycling in general and P in particular (Malik et al., 2013; Masto et al., 2013; Tamilselvi et al., 2015). Organic matter contributes 20–80% to the organic phosphorous in soil (Richardson, 1994), which in turn is hydrolyzed by phosphatases – enzymes of plant or microbial origin – to become plant available P (Tarafdar and Claassen, 1988). Not only does the mineralization of organic manure supplies available P for plant uptake, it also plays a significant role in mobilization of native P forms through an array of mechanisms. For instance, organic anions evolved during manure decomposition, metal complexation or dissolution reactions mediate release of P from exchange sites (Bolan et al., 1994; Iyamuremye and Dick, 1996). Also, the addition of organic matter serves as a substrate for microbial proliferation that aides in changing the dynamics of P (both organic and inorganic forms) in the rhizosphere thereby positively affecting root architecture and biological properties such as root released phosphatases or phosphatases of microbial origin or both (Gichangi et al., 2009; Richardson et al., 2011; Guan et al., 2012; Malik et al., 2013). The effectiveness of added organic manures on microbial activity can be ascertained by assessing its influence on pertinent changes in soil properties such as pH, soil enzymatic activities, microbial biomass and its role in P mobilization and assimilation. The assay of soil enzymatic activities could provide an early and sensitive indication of changes induced by management strategies such as organic manuring, green manuring, crop residue incorporation, tillage interventions, herbicide application etc. (Dick et al., 1988; Nannipieri, 1994; Aparna et al., 2014; Tamilselvi et al., 2015). Enzyme activity coupled with measurements of other relevant biological and biochemical parameters (e.g., soil respiration, microbial carbon biomass, soil pH etc.) provides indication on the extent of biological activity in soil. Because of the inherent complexity of multiple co-existing soil processes, it is, however, challenging to quantify the net contribution of each of these factors to plant P-availability under specific production systems.

The proclaimed effectiveness of organic management in enhancing P availability could only be determined by systematic comparison with conventional management systems under field conditions. Such comparative studies need to also consider the minimum time required for organic systems to become fully functional. Despite the fact that P availability in soils is of high scientific interest, systematic long-term comparisons of factors contributing to P availability in organic and conventional farming systems are lacking. In this study, we compared soil biological activities pertaining to P availability at key crop growth stages in agricultural plots that were subject to continuous organic and conventional management for 7 years. The study was conducted within the framework of a long-term farming systems comparison trial in Vertisols of Central India, where soybean (*Glycine max*) – wheat (*Triticum aestivum*) is a predominant cropping system. We hypothesized that biological activity in soils of organic production systems plays a significant role in P mobilization in a soybean–wheat cropping system. The specific objective of this study was to monitor changes in and synergies among soil biological parameters contributing to P availability such as soil dehydrogenase activity (DHA), β-glucosidase (βGL), acid phosphatase (ACP) and alkaline phosphatase (ALP) activities, soil microbial respiration (SR), substrate induced respiration (SIR) and soil microbial biomass carbon (MBC) content at key growth stages of soybean and wheat crops.

MATERIALS AND METHODS

Site and Trial Description

This study was conducted on the field site of the long term farming systems comparison (SysCom) trial running since 2007 in the Nimar valley of Madhya Pradesh state in central India. The trial site is located at an altitude of 250 m above sea level (22°8′30.28″N; 75°37′48.97″E) in a subtropical (semi-arid) climate with an average temperature of 25°C (temperature range 05–48°C) of which the maximum temperature occurs during May/June and minimum temperature during January/February. This region receives an average precipitation of 800 mm, most of which comes during monsoon period from June to September (**Figure 1**). The experimental site belongs to Vertisols (Fine, iso-hyperthermic, montmorillonitic, Typic Haplusterts) and the pertinent soil characteristics at the start of the experiment in 2007 were pH 8.7, organic carbon content 5.0 gkg^{-1}, clay content 600 gkg^{-1}, $CaCO_3$ 55 gkg^{-1}, and available (Olsen's) P content of 7.0 mg kg^{-1} (Forster et al., 2013). Cotton/ soybean–wheat is the predominant cropping pattern in Nimar valley, though farmers also grow other crops such as sugarcane, vegetables, fodder, and pulses.

As described by Forster et al. (2013), the field site of SysCom trial was under conventional management until December 2006, when a test crop of unfertilized wheat was grown to assess the homogeneity of the terrain before setup of the trial. The trial consists of four treatments – two organic farming system, i.e., organic (BIOORG) and biodynamic (BIODYN) and two conventional farming systems, i.e., conventional (CON) and conventional including Bt-cotton (CONBtC). These management systems are replicated four times in a randomized block design in two stripes of plots with gross plot size of 16 m × 16 m and net plot size of 12 m × 12 m. While designing the treatment compositions, due consideration was given to prevalent practices of local farmers as well as standard recommendations. As a rule of thumb, organic management systems are implemented according to the standards prescribed by International Federation of Organic Agriculture movements (IFOAM, 2006) and conventional management is carried out in line with the recommendations of Indian Council of Agricultural Research, with slight adaptations to suit the prevailing local situations (Forster et al., 2013). The nutrient inputs in organically managed plots are mainly supplied by compost, castor cake, rock phosphate, and farm yard manure (FYM), while in conventional management systems, inorganic fertilizers are applied in the form of urea, diammonium phosphate (DAP), Single super phosphate (SSP) and muriate of Potash. It is noteworthy that following the principle of good agricultural practices (and practice of local farmers) every alternative year conventional plots also receive a basal application of 4 t ha^{-1} FYM. This dose of FYM was applied in the previous year (2013). In 2014, conventional system (Soybean + wheat) received a total of 178 kg N ha^{-1}, 78 kg P ha^{-1}, and 88 kg K ha^{-1} from synthetic mineral fertilizers; whereas organic system received a total of 151 kg N ha^{-1}, 79 kg P ha^{-1}, and 173 kg K ha^{-1}from organic inputs. Soybean crop (variety JS 93-05; seed rate 80 kg ha^{-1}) was applied with a basal

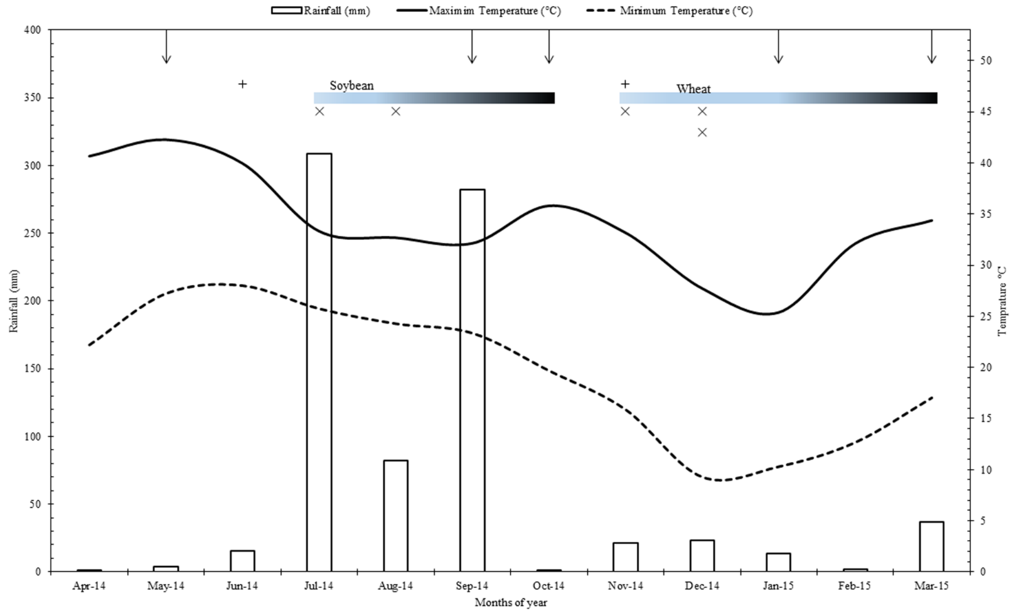

FIGURE 1 | Rainfall, minimum and maximum temperatures of the experimental site during the period of study (2014–2015). Where, vertical arrows from top horizontal axis represent the time of sampling and gradient bars indicate the crop growth periods for Soybean and wheat; +, Farmyard + rock phosphate and compost application in organic systems; x, fertilizers application in conventional systems.

application of 45 kg P ha^{-1} and 52.5 kg K ha^{-1} from SSP and MOP, respectively, in conventional system. Single dose of 28.5 kg N ha^{-1} from urea was applied at 19 DAS. In organic system, 2.5 t ha^{-1} of FYM and 2.4 t ha^{-1} of acidulated rock phosphate was applied and incorporated in soil by bullock drawn harrow at 34 days before sowing of soybean. Application of FYM and acidulated rock phosphate provided 47 kg N ha^{-1}, 33 kg P ha^{-1}, and 47 kg K ha^{-1} to wheat crop in organic system. In wheat [variety HI-1544 (Purna), seed rate 100 kg ha^{-1}], after soybean, basal application of 33 kg P ha^{-1} and 35 kg K ha^{-1} was applied with SSP and MOP, respectively, in conventional. A total of 149 kg of N ha^{-1} was applied in two identical splits at 19 and 43 DAS. Three days before sowing 13 t ha^{-1} of compost was applied to organic system and incorporated in soil by bullock drawn harrow, which provided 105 kg N ha^{-1}, 47 kg P ha^{-1}, and 126 kg K ha^{-1}. All the cultural management practices such as weed and pest management were followed as per standard norms prescribed for organic and conventional systems (Forster et al., 2013).

Soil Sampling and Analysis

Organic (BIOORG) and conventional (CON) system plots were sampled during soybean and wheat crops at five different time points, i.e., from fallow land before sowing of soybean, R2 stage of soybean, before sowing of wheat, panicle initiation stage of wheat and after harvest of wheat (**Figure 1**). R2 stage of soybean and panicle initiation stage of wheat are of high reproductive significance and thus important for crop productivity. Each plot was sampled to 0–20 cm depth from six random locations and collected samples were pooled for analysis. DHA was assessed through the reduction of 2,3,5- triphenyltetrazolium chloride (TTC) to triphenylformazan (TPF) using colorimetric procedure (Shimadzu UV-VS, Model- 1800) of Tabatabai (1994) and expressed as μg triphenylformazan g^{-1} soil h^{-1} (Klein et al., 1971). βGL activity was determined using *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05M) as a substrate (Sinsabaugh et al., 1999) and the amount of *p*-nitrophenol released was determined spectrophotometrically at OD_{420} and expressed as μg *p*-nitrophenol g^{-1} soil h^{-1} (Tabatabai, 1994). ACP and ALP were assayed by the standard method of Tabatabai and Bremner (1969) in acetate buffer (pH 5.4) and borax-NaOH buffer (pH 9.4), respectively, using *p*-nitrophenyl phosphate as a substrate. Soil pH was determined in a soil: water ratio of 1:2.5 with intermittent stirring for 30 min and feeding directly to a pH meter (Baruah and Barthakur, 1999). SR was determined by quantifying the carbon dioxide released in the process of microbial respiration during 10 days of incubation (Anderson and Domsch, 1990). SIR was determined by quantifying the carbon dioxide released in the process of microbial respiration during 2 h incubation after adding (0.0625 g) glucose and (2.5 g) talc to soil (Anderson and Domsch, 1978). Microbial biomass-Carbon was estimated by employing the fumigation-extraction procedure of Vance et al. (1987) and was calculated from the relationship $Bc = Fc/Kc$, where Fc is the difference between extractable carbon from fumigated soil and non-fumigated soil; Kc is conversion factor, which is 0.45 and the value has been expressed in mg C kg^{-1} soil (Joergensen and Mueller, 1996). Olsen P was extracted with 0.5 M sodium bicarbonate (pH 8.5) in 1:5 ratio of soil to extractant and shaken for 30 min at 150 rpm (Olsen et al., 1954). After filtration of suspension, phosphorus concentration in the extract was estimated colorimetrically by ascorbic acid reductant method (Watanabe and Olsen, 1965). For P content of seed and straw, samples collected from soybean and wheat crops were air-dried and kept in an oven at 65°C till constant weight. Upon grinding the samples were passed through 0.5 mm sieve and digested in acid mixture of HNO_3:$HClO_4$, 5:4 ratio. The phosphorus concentration in the digest was determined colorimetrically using vanadomolybdate yellow color method. The seed and straw yield of each net plot was recorded and converted to kg ha^{-1}.

Statistical Analysis

The data was analyzed by using SAS statistical software (ver.9.2; SAS Institute., Cary, NC, United States). For microbiological parameters and available P content, three way analysis was carried out involving treatments (Organic, conventional) crops (Soybean, wheat) and periods of sampling and their interactions as fixed factors. The significant differences between means were identified using Fisher least significant differences (LSD) and Tukeys multiple comparison tests at $P = 0.05$. For crop yield and uptake parameters, one way analysis of variance (ANOVA) was carried out using the ANOVA procedure in SAS enterprise guide 4.2 and means separated with LSD and Tukeys multiple comparison tests. In order to obtain a comprehensive picture of potential synergistic interactions among the observed biological and microbiological parameters, a Principle Component Analysis (PCA) was carried out. Principle components thus constructed allowed to define which original variables are responsible for the mean difference between systems. PCA was performed using JMP (©SAS Institute Inc.) (Goupy and Creighton, 2007).

RESULTS

First objective of this study was to monitor changes in soil biological properties pertaining to P cycling in organic and conventional management systems. The assessed soil microbiological and chemical parameters showed considerable variation across systems and crop growth stages. Soil DHA did significantly vary between organic and conventional systems at sowing under soybean cropping. Significant increase of up to 16.3% (66.3 μg triphenylformazon g^{-1} soil 24 h^{-1}) and 8.7% (58.7 μg triphenylformazon g^{-1} soil 24 h^{-1}) was observed in organic and conventional systems, respectively, at R2 stage as compared to sowing (**Table 1**). At R2 stage, organic management registered 12.9% increase in DHA over conventional system. At harvest, there was a significant decline in DHA in both the agricultural systems as compared to its activity at R2 stage and also it showed significant variation between the agricultural management systems with higher DHA in organically managed systems. In wheat crop, DHA was significantly higher by 49% (100.6 μg triphenylformazon g^{-1} soil 24 h^{-1}) in organic management as compared to the conventional system (71.2 μg triphenylformazon g^{-1} soil 24 h^{-1}). DHA was relatively higher at active crop growth stages in both soybean and wheat while

TABLE 1 | Effect of organic and conventional agricultural management practices on soil dehydrogenase, β-glucosidase, acid phosphatase, and alkaline phosphatase activities at different periods of sampling in soybean and wheat crop.

	Dehydrogenase (μg triphenylformazon g^{-1}soil 24 h^{-1})		β-Glucosidase (μg p-nitrophenol g^{-1} soil h^{-1})		Acid phosphatase (μg p-nitrophenol g^{-1} soil h^{-1})		Alkaline phosphatase (μg p-nitrophenol g^{-1} soil h^{-1})	
Treatments (T)	**Organic**	**Conventional**	**Organic**	**Conventional**	**Organic**	**Conventional**	**Organic**	**Conventional**
Treatment mean	66.9 ± 3.5^{a}	57.1 ± 2.8^{b}	216.1 ± 6.5^{a}	188.7 ± 11.9^{a}	198.6 ± 14.5^{a}	180.4 ± 14.5^{a}	413.4 ± 11.9^{a}	332.6 ± 22.8^{b}
Period of sampling (PS)								
Soybean								
At sowing	57.0 ± 1.7^{aB}	54.0 ± 4.0^{aB}	141.3 ± 5.6^{aB}	132.3 ± 9.0^{aB}	182.9 ± 15.0^{aA}	164.6 ± 12.4^{aA}	400.7 ± 23.6^{aA}	360.8 ± 13.5^{bA}
50 DAS	66.3 ± 3.1^{aA}	58.7 ± 2.6^{bA}	215.4 ± 4.6^{aA}	186.8 ± 4.5^{bA}	245.7 ± 5.9^{aB}	225.4 ± 8.0^{bB}	500.7 ± 18.6^{aA}	459.8 ± 29.2^{aB}
Harvest	56.6 ± 4.8^{aB}	52.0 ± 2.5^{aB}	$155.9 \pm 6.1^{a\ B}$	$123.4 \pm 7.6^{a\ B}$	226.1 ± 21.6^{aB}	214.1 ± 19.9^{aB}	465.8 ± 7.2^{aA}	374.7 ± 25.6^{bA}
Mean of soybean	60.0^{a}	54.2^{b}	170.9^{a}	147.5^{a}	218.2^{a}	201.4^{a}	455.7^{a}	398.4^{b}
Wheat								
60 DAS	100.6 ± 5.3^{aA}	71.2 ± 3.5^{bA}	337.8 ± 5.6^{aA}	292.1 ± 11.0^{bA}	$184.5 \pm 16.5^{a\ a}$	175.2 ± 17.5^{aA}	412.3 ± 0.0^{aB}	318.9 ± 15.9^{bA}
Harvest	54.2 ± 2.5^{aB}	49.3 ± 1.2^{bB}	230.1 ± 10.5^{aB}	208.9 ± 27.3^{aB}	$154.0 \pm 13.7^{a\ A}$	123.0 ± 14.9^{bC}	287.6 ± 10.2^{aC}	148.9 ± 30.0^{bC}
Mean of wheat	77.4^{a}	60.25^{b}	283.95^{a}	250.5^{a}	169.5^{a}	149.1^{a}	349.95^{a}	233.9^{b}

Data are mean values of four replicates; values followed by the same small letters are not significantly different among organic and conventional practices at $P = 0.05$ (horizontal comparison). Similarly values followed by the same capital letters are not significantly different between different periods of sampling (vertical comparison). Values under ANOVA are probabilities (P-values) of the source of variations.

it decreased at harvest. Organically managed system exhibited higher DHA activity throughout the experimental period, which was on an average 17.2% higher than conventional system (**Table 1**).

Significantly higher βGL activity under organic management system was observed at R2 stage of soybean with a difference of 15.3% (215.4 μg p-nitrophenol g^{-1} soil h^{-1}) to conventional management system (**Table 1**). From sowing to R2 stage of soybean, βGL activity saw a significant increase of 52.4% in organic (215.4 μg p-nitrophenol g^{-1} soil h^{-1}) and 41.2% (186.8 μg p-nitrophenol g^{-1} soil h^{-1}) in conventional system and there was a significant decline at harvest. At panicle initiation stage of wheat, βGL activity was 15.7% (337.8 μg p-nitrophenol g^{-1} soil h^{-1}) higher in organic management system than conventional management system.

Acid phosphatase activity was significant at R2 stage of soybean and at harvest of wheat. In contrast, ALP activity was significantly higher in organic systems as compared to conventional at all the sampling times except for R2 stage of soybean (**Table 1**). Considering the overall average of the soybean–wheat system 24% higher (413.4 μg p-nitrophenol g^{-1} soil h^{-1}) ALP activity was recorded under organic management compared to the conventional management. MBC increased during the active crop growth stages and decreased toward harvest of soybean and wheat. At each of the sampling points, MBC tended to be higher under organic management than conventional, but the differences were never significant (**Table 2**). Similar was the case of SIR, which increased from second sampling (R2 stage) onward and decreased at harvest of wheat. Organic system exhibited slightly higher MBC than conventional system throughout the experiment but did not attain the level of significance (**Table 2**). SR was higher in organically managed soil before sowing of soybean, at R2 stage and at harvest of soybean. Whereas, in case of wheat, organic and conventional systems were statistically not different for SR at both the sampling points (**Table 2**). Within each management system, SR did not exhibit a major change during the different sampling points except for a significant decline at the harvest of wheat.

Soil pH did not differ significantly between organic and conventional management practices in soybean at any stage, however, a significant decline in both the management systems was observed at harvest of soybean (**Table 2**). The available phosphorus content was highest at R2 stage of soybean, irrespective of the production system. Though the availability of P tended to be slightly higher under organic management at different sampling points, the differences were not statistically significant (**Table 2**). Seed yield of soybean was statistically similar under organic (1902 kg ha^{-1}) and conventional management (1848 kg ha^{-1}). Similarly, soybean straw yield was also comparable in organic (1756 kg ha^{-1}) and conventional system (1723 kg ha^{-1}). However, in case of wheat, conventional system produced significantly higher seed and straw yield than organic.

The results of PCA analysis provided a comprehensive picture of parameters that work synergistically in each management system. In the bi-plot (**Figure 2**), length of the vector corresponding to a particular soil parameter demonstrates the

TABLE 2 | Effect of organic and conventional agricultural management practices on microbial biomass Carbon, substrate induced respiration, microbial respiration, pH, and available phosphorous at different periods of sampling in soybean and wheat crop.

	Microbial biomass carbon (mg C Kg^{-1} soil)		Substrate induced respiration (mg CO_2 Kg^{-1} h^{-1})		Microbial respiration (mg CO_2 Kg^{-1} day^{-1})		pH		Available phosphorous (μg g^{-1} soil)	
Treatments (T)	**Organic**	**Conventional**	**Organic**	**Conventional**	**Organic**	**Conventional**	**Organic**	**Conventional**	**Organic**	**Conventional**
Treatment mean	311.7 ± 22.8^{a}	294.2 ± 20.2^{a}	19.1 ± 1.4^{a}	17.5 ± 1.3^{a}	16.5 ± 0.5^{a}	15.2 ± 0.6^{b}	8.02 ± 0.1^{a}	7.97 ± 0.2^{a}	5.9 ± 0.1^{a}	5.6 ± 0.1^{b}
Period of sampling (PS)										
Soybean										
At sowing	270.8 ± 25.0^{aA}	250.0 ± 13.6^{aA}	13.8 ± 2.3^{aA}	12.6 ± 1.2^{aA}	16.3 ± 0.7^{aA}	14.6 ± 0.5^{bA}	8.09 ± 0.0^{aA}	8.05 ± 0.1^{aA}	5.4 ± 0.1^{aA}	5.2 ± 0.2^{aA}
50 DAS	391.7 ± 21.5^{aB}	362.5 ± 25.0^{aB}	22.2 ± 1.2^{aB}	20.4 ± 1.4^{aB}	17.4 ± 0.5^{aA}	15.2 ± 0.9^{bA}	8.07 ± 0.1^{aA}	8.08 ± 0.2^{aA}	7.6 ± 0.1^{aB}	7.3 ± 0.2^{aB}
Harvest	320.8 ± 25.0^{aC}	308.33 ± 21.5^{aC}	19.8 ± 1.2^{aB}	18.0 ± 1.4^{aB}	16.4 ± 0.7^{aA}	15.1 ± 0.7^{bA}	7.88 ± 0.2^{aB}	7.91 ± 0.2^{aB}	6.1 ± 0.2^{aC}	5.8 ± 0.1^{aA}
Mean of soybean	327.8^{a}	306.9^{a}	18.6^{a}	17.0^{a}	16.7^{a}	14.9^{b}	8.01^{a}	8.01^{a}	6.4^{a}	6.1^{a}
Wheat										
60 DAS	325.0 ± 28.7^{aB}	312.5 ± 16.0^{aB}	22.2 ± 1.2^{aB}	20.4 ± 1.4^{aB}	16.9 ± 0.4^{aA}	16.4 ± 0.3^{aA}	8.12 ± 0.5^{aA}	7.83 ± 0.1^{bC}	5.6 ± 0.1^{aC}	5.1 ± 0.1^{aA}
Harvest	250.0 ± 13.6^{aA}	237.5 ± 25.0^{aA}	17.4 ± 1.2^{aA}	16.2 ± 1.2^{aA}	15.6 ± 0.4^{aB}	15.0 ± 0.5^{aB}	8.07 ± 0.1^{aA}	8.04 ± 0.2^{aA}	5.0 ± 0.1^{aA}	4.8 ± 0.1^{aC}
Mean of wheat	287.5^{a}	275^{a}	19.8^{a}	18.3^{a}	16.25^{a}	15.7^{a}	8.1^{a}	7.9^{a}	5.3^{a}	5.0^{a}

Data are mean values of four replicates; values followed by the same small letters are not significantly different among organic and conventional practices at P = 0.05 (horizontal comparison). Similarly values followed by the same capital letters are not significantly different between different periods of sampling (vertical comparison). Values under ANOVA are probabilities (P-values) of the source of variations.

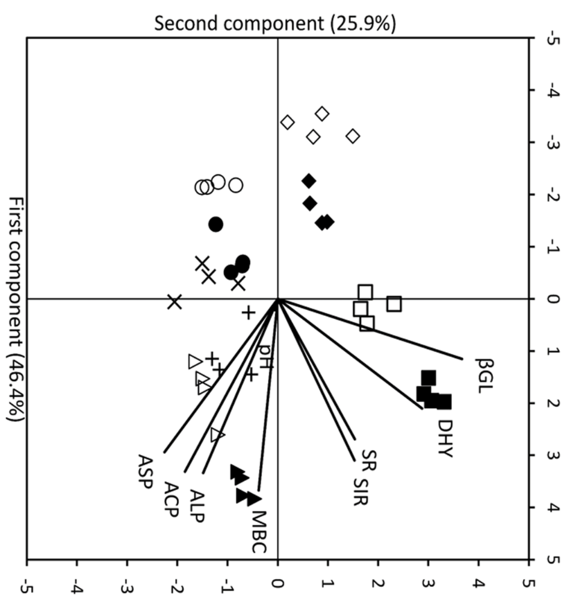

FIGURE 2 | Bi-plot for the principal component analysis of organic and conventional soybean and wheat management systems at five different times of sampling: Before sowing of Soybean (○ CONV, ●ORG), 50 DAS (△ CONV, ▲ ORG), before sowing of wheat (× CONV, + ORG), 60 DAS (☐ CONV, ■ ORG), and after wheat harvest (◇ CONV, ◆ ORG). Soil pH (pH), microbial biomass carbon (MBC), acid phosphatase (ACP), alkaline phosphatase (ALP), arylsulphatase (ASP), β-glucosidase (βGL), dehydrogenase (DHA), microbial soil respiration (SR), and substrate induced respiration (SIR).

extent of relative contribution of that parameter. Whereas, the proximity of a vector to a symbol cluster indicates the association of that biological parameter to the particular farming system and sampling time represented by that symbol cluster. Cumulative variability of 84.2% was captured by first three principal components (PC) (**Table 3**). Distinguished presence of farming systems' clustered replicates in different quadrates indicated the extent of activities of variables at different sampling times (**Figure 2**). Dissociation between systems and variables at soybean sowing, wheat harvest and wheat sowing (only conventional) clearly came out in PCA from the presence of respective points in 2nd and 3rd quadrate, which are aloof from vectors of variables. In organic systems, the main active variable selected by PCA was the MBC at R2 stage of soybean and DHA was the main active variable at panicle initiation stage of wheat (**Figure 2**). No such association of a particular variable at active crop growth stages of soybean and wheat was found in conventional system. The first PC explained 46.6% of variability with major contribution of MBC, ACP, and ALP. In 2nd component major contribution comes from βGL and DHA which explained variability of 25.9%. Soil pH was the only major contributor for the PC3 and explained the variability of 11.9%.

DISCUSSION

The overall hypothesis of this study was supported by finding of higher biological activity in organic systems that resulted

TABLE 3 | Eigenvectors corresponding to principal components including eigenvalues and cumulative proportion variance of measured variables.

Variables	**PC**		
	1	**2**	**3**
pH	0.03	−0.01	0.95
MBC	0.45	−0.06	−0.01
ACP	0.40	−0.30	−0.07
ALP	0.41	−0.24	0.05
ASP	0.36	−0.37	0.11
βGL	0.14	0.60	0.07
DHA	0.26	0.47	0.14
SR	0.33	0.25	−0.03
SIR	0.38	0.25	−0.23
Eigenvalue	4.18	2.33	1.07
Cumulative proportion	46.4	72.3	84.2

in attaining P availability equivalent to conventional systems. Soil microbiological parameters such as DHA, βGL, ACP, ALP, SIR, SR, and MBC were in general higher in soil of plots under 7 years of organic management compared to those under conventional systems, particularly at key growth stages of both soybean and wheat crops. Soil enzymes have been suggested as potential indicators of soil quality because of their ease of measurements, relationship to belowground microbiological processes and rapid response to changes in agricultural management (Dick et al., 1996; Dick, 1997; Jimenez et al., 2002). Measurement of soil enzyme activities also provides an integrative response to changes in soil chemical, physical and biological characteristics under different management induced perturbations and is used to monitor the effects of different agricultural management strategies on long-term productivity (Doran and Parkin, 1994; Ndiaye et al., 2000; Acosta-Martinez et al., 2007). These measurements also provide credible information on the key reactions that participate in the rate limiting steps in the decomposition of soil organic matter and nutrient transformation in soils and are thus of high relevance in understanding P availability under different management systems. The soil enzyme activities measured in this study increased from sowing to R2 stage in soybean and to panicle initiation stage in wheat crop and again declined toward harvest. This increase in soil enzyme activities during active crop growth stages can be ascribed to increased rhizo-deposition (Gregory, 2006; Mandal et al., 2007; Nayak et al., 2007; Masto et al., 2013; Tamilselvi et al., 2015). The higher enzyme activity in organic agricultural system can also be attributed to enhanced nutrient availability from added organic inputs, increased root exudation owing to improved crop growth and conducive environment for microbial proliferation (Burns et al., 2013; Tamilselvi et al., 2015). PCA results showed that DHA was main contributing factor in organic systems at panicle initiation stage of wheat. Dehydrogenase is an oxidoreductase enzyme that is present only in viable cells and measurement of DHA provides an index of endogenous soil microbial activity as its assay involves no addition of substrate that preferentially stimulates any particular group of soil microorganisms (Biederbeck et al., 2005). For this reason, DHA assay has been used as a potential soil quality indicator to discriminate changes under different agricultural management systems (Kandeler et al., 1999; Aseri and Tarafdar, 2006; Aparna et al., 2014).

Similarly, βGL is involved in decomposition of cellulose compounds and is synthesized by soil microorganisms in the presence of suitable substrates. Therefore, it has been used as sensitive indicator of microbially mediated soil processes (Sinsabaugh, 1994; Lagomarsino et al., 2009; Stott et al., 2010). Phosphatase activity in the soils may originate either from plant roots or from microorganisms such as fungi and bacteria (Tarafdar and Chhonkar, 1979; Tarafdar et al., 1988; Dinkelaker and Marschner, 1992) and changes in its activity could indicate changes in the quantity and quality of soil phosphoryl substrates (Rao and Tarafdar, 1992). Apart from creating conducive environment for increased biological activity, organic amendments are rich in microbial biomass and may also contain intra- and extracellular enzymes that stimulate microbial activity in soil (Liang et al., 2005; Tejada et al., 2006). Our findings are consistent with earlier studies that showed an increase in enzyme activities with the application of organic amendments (Marinari et al., 2006; Tejada et al., 2006; Aparna et al., 2014).

Generally, organic inputs increase C and energy availability to microorganisms, thereby stimulating indigenous soil microbial biomass and activity, especially in C-depleted agricultural soils. In a long-term study conducted under temperate environmental conditions, Fließbach and Mäder (2000), found 45–64% higher microbial biomass in bio-dynamic farming systems than conventional systems after18 years of respective crop management. In contrast, our results show only an average increase of about 6% MBC under organic management after 7 years of experimentation, while the differences are non-significant at individual sampling points. This indicates that due to higher turnover rates under tropical environments (as in our study), 7 years is probably not a long enough period to see clearly distinguishable differences in MBC. Moreover, owing to the concept of good agricultural practices, conventionally managed plots in this field trial also receives four tons of FYM every alternate year (Forster et al., 2013), which contributes to MBC in conventional plots and hence might have acted as a confounding factor minimizing differences among productions systems. Nevertheless, PCA results showed that MBC was the main factor contributing to biological activities at R2 stage of soybean in organic systems. Soil microbial respiration rate gives an indication of microbiological activity in the soil and is influenced by carbon availability to microorganisms in the soil environment. We found higher rates of microbial respiration in organically amended soils, which could be attributed to greater labile fractions of organic matter in the added organic manures (Tu et al., 2006; Chinnadurai et al., 2014; Tamilselvi et al., 2015; Hernández et al., 2016). Similarly, SIR, another soil quality indicator that provides us information on the metabolic and physiological state of soil microorganisms (Anderson, 1994), tended to be higher under organic management (Chinnadurai et al., 2014; Tamilselvi et al., 2015). Moreover, both SR and SIR were found to be significantly higher at active crop growth stages that could be attributed to increased rhizo-deposition which is conducive for microbial proliferation (Mandal et al., 2007; Li et al., 2012; Masto et al., 2013; Tamilselvi et al., 2015).

Soil pH is considered an important factor influencing P availability in soils and it could play a crucial role in alkaline soils of our experimental site. However, in this study the differences in pH among organic and conventional systems on an average were not significant enough to exert a major influence *per se*. The most interesting observation in this regard was the dip in pH at the harvest of soybean, which recovered in organic systems (8.12) in the subsequent sampling (panicle initiation stage of wheat) but not in conventional (7.83). The reduction in pH at the harvest of soybean is plausible as the leguminous plants are known to reduce soil pH (Yan et al., 1996; Opala et al., 2012). However, the observed differences in pH at panicle initiation stage of wheat seem strongly influenced by management practices. The organic systems received a basal dose of FYM based compost at the planting of wheat, which seems to have contributed to quick recovery in pH (Whalen et al., 2000). Whereas, conventional systems received a basal dose of mineral P and K fertilizers (SSP and MOP) at sowing of wheat and two split doses of N (Urea) at 19 and 43 DAS, which might have resulted in a lower pH. Use of acidifying inorganic mineral fertilizers over considerably longer periods is known to result in a decline in soil pH (Birkhofer et al., 2008), which could in turn affect aggregate stability and loss of organic matter (Mäder et al., 2002; Mikha and Rice, 2004). Inputs of organic manures applied every alternate year to conventional plots in this study might be an important contributing factor in slowing down the acidification of soil over longer term.

On an average, P availability in the soil under organic management tended to be higher (5.9 $\mu g\ g^{-1}$) than that under conventional management (5.6 $\pm$ 0.1 $\mu g\ g^{-1}$). However, at any particular sampling time, differences in P availability among the two management systems were not statistically significant. It is noteworthy that despite the application of mineral P in conventional plots at sowing of wheat, the availability of P tended to be slightly lower than that under organic management. The values of P availability at panicle initiation stage of wheat under organic (5.6 $\mu g\ g^{-1}$) and conventional (5.1 $\mu g\ g^{-1}$) management indicate that most of the P applied to conventional plots in the form of mineral fertilizer was either utilized by the crop or fixed by the soil. Since yield of wheat was considerably higher under conventional management, it is plausible that the P applied at sowing was taken up by the crop by panicle initiation stage. Comparing the P availability among two management systems at all the five sampling times, we can conclude that P availability was not a limiting factor for organic at any of these time points. However, utilization of available P by crop plants depends on several factors and N availability could be one of them (Riar and Coventry, 2012). Since soybean can symbiotically assimilate atmospheric nitrogen, probably it had relatively higher capability of utilizing available P compared to wheat. Therefore, soybean yield under organic management was equivalent to conventional management, which was not the case of wheat. Further investigations would be needed to identify the factors responsible for yield difference in wheat, however, yield is a complex trait influenced by multiple factors discussion of which is beyond the scope of this study.

From the findings of this study, we conclude that owing to higher biological activity, organically managed agricultural soils could attain equivalent or higher P availability than conventionally managed soils receiving regular inputs of mineral P fertilizers. These results are of particular relevance to alkaline vertisols, wherein sorption and precipitation are important influencing factors in determining the availability of P. These findings also carry a high global applicability, for instance, P-fixing soils are widely prevalent in Africa, where P-inputs through mineral fertilizers are ineffective. Organic management over a considerable time period could support in building up fertility and enhancing P availability in these soils. Moreover, it also offers a suitable alternative to resource poor small holder farmers of developing countries who cannot afford the expensive mineral fertilizers.

AUTHOR CONTRIBUTIONS

NB, GB, and SI conceived the project. NB conducted the field study and lab work. AkR, MS, and SS provided scientific support for the lab work. NB, AkR, MS, and AmR analyzed the data. NB, AkR, AmR, and GB wrote the manuscript and all authors revised the manuscript.

FUNDING

This study was conducted in the framework of long-term farming systems comparison in the tropics (SysCom) program, which is financially supported by Biovision Foundation for Ecological Development, Coop Sustainability Fund, Liechtenstein Development Service (LED) and the Swiss Agency for Development and Cooperation (SDC).

REFERENCES

Acosta-Martinez, V., Cruz, L., Ramirez, D. S., and Alegria, L. P. (2007). Enzyme activities as affected by soil properties and land use in a tropical watershed. *Appl. Soil Ecol.* 35, 35–45. doi: 10.1016/j.apsoil.2006.05.012

Alam, M. M., and Ladha, J. K. (2004). Optimizing phosphorus fertilization in an intensive vegetable–rice cropping system. *Biol. Fertil. Soils* 40, 277–283. doi: 10.1007/s00374-004-0778-7

Anderson, J. P. E., and Domsch, K. H. (1978). A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biol. Biochem.* 75, 43–48. doi: 10.1016/0038-0717(78)90099-8

Anderson, T. H. (1994). "Physiological analysis of microbial communities in soil: application and limitations," in *Beyond the Biomass*. eds K. Ritz, J. Dighton, and K. E. Giller (Exeter: Wiley-Sayce), 67–76.

Anderson, T. H., and Domsch, K. H. (1990). Application of eco-physiological quitients (qCO2 and qD) on microbial biomasses from soils of different cropping histories. *Soil Biol. Biochem.* 25, 393–395. doi: 10.1016/0038-0717(93)90140-7

Aparna, K., Pasha, M. A., Rao, D. L. N., and Krishnaraj, P. U. (2014). Organic amendments as ecosystem engineers: microbial, biochemical and genomic evidence of soil health improvement in a tropical arid zone field site. *Ecol. Eng.* 71, 268–277. doi: 10.1016/j.ecoleng.2014.07.016

Aseri, G. K., and Tarafdar, J. C. (2006). Fluoresciencediacetate: a potential biological indicator for arid soils. *Arid Land Res. Manag.* 20, 87–99. doi: 10.1007/s00248-011-9867-y

Aulakh, M. S., Kabba, B. S., Baddesha, H. S., Bahl, G. S., and Gill, M. P. S. (2003). Crop yields and phosphorus fertilizer transformations aft er 25 years of applications to a subtropical soil under groundnut based cropping systems. *Field. Crops Res.* 83, 283–296. doi: 10.1016/S0378-4290(03)00078-9

Bai, Z., Li, H., Yang, X., Zhou, B., Shi, X., Wang, B., et al. (2013). The critical soil P levels for crop yield, soil fertility and environmental safety in different soil types. *Plant Soil* 372, 27–37. doi: 10.1007/s11104-013-1696-y

Baruah, T. C., and Barthakur, H. P. (1999). *A Text Book of Soil Analysis*. New Delhi: Vikas Publishing House Pvt. Ltd.

Basamba, T. A., Barrios, E., Amezquita, E. I., Rao, M., and Singh, B. R. (2006). Tillage effects on maize yield in a Colombian savanna Oxisol: soil organic matter and P fractions. *Soil Tillage Res.* 91, 131–142. doi: 10.1016/j.still.2005.11.010

Biederbeck, V. O., Zetner, R. P., and Campbell, C. A. (2005). Soil microbial populations and activities as influenced by legume green fallow in a semi-arid climate. *Soil Biol. Biochem.* 37, 1775–1784. doi: 10.1016/j.soilbio.2005.02.011

Birkhofer, K., Bezemer, T. M., Bloem, J., Bonkowski, M., Christensen, S., Dubois, D., et al. (2008). Long-term organic farming fosters below and aboveground biota: implications for soil quality, biological control and productivity. *Soil Biol. Biochem.* 40, 2297–2308. doi: 10.1016/j.soilbio.2008.05.007

Bolan, N. S., Naidu, R., Mahimairaja, S., and Baskaran, S. (1994). Influence of low molecular- weight organic acids on the solubilization of phosphates. *Biol. Fertil Soils* 18, 311–319. doi: 10.1590/S1517-838246246220131102

Brady, N. C., and Weil, R. R. (2008). *The Nature and Properties of Soils*, 4th Edn. Upper Saddle River, NJ: Prentice Hall.

Burns, R. G., DeForest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E., Wallemstein, M. D., et al. (2013). Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biol. Biochem.* 32, 1547–1559. doi: 10.1016/j.soilbio.2012.11.009

Chinnadurai, C., Gopalaswamy, G., and Balachandar, D. (2014). Impact of long-term organic and inorganic nutrient managements on the biological properties and eubacterial community diversity of the Indian semi-arid Alfisol. *Arch. Agron. Soil Sci.* 60, 531–548. doi: 10.1080/03650340.2013.803072

Ciampitti, I. A., García, F. O., Picone, L. I., and Rubi, G. (2011). Phosphorus budget and soil extractable dynamics in field crop rotations in mollisols. *Soil Sci. Soc. Am. J.* 75, 131–142. doi: 10.2136/sssaj2009.0345

Condron, L. M., Turner, B. L., and Cade-Menum, B. J. (2005). "Chemistry and dynamics of soil organic phosphorus," in *Phosphorus: Agriculture and the Environment*, eds J. T. Sims and A. N. Sharpley (Madison, WI: American Society of Agronomy), 87–121.

Cordell, D., Drangert, J. O., and White, S. (2009). The story of phosphorus: global food security and food for thought. *Glob. Environ. Change* 19, 292–305. doi: 10.1016/j.gloenvcha.2008.10.009

Damodar Reddy, D., Subba Rao, A., Sammi Reddy, K., and Takkar, P. N. (1999). Yield sustainability and phosphorus utilization in soybean-wheat system on vertisols in response to integrated use of manure and fertilizer phosphorus. *Field Crops Res.* 62, 181–190. doi: 10.1016/S0378-4290(99)00019-2

Dick, R. P. (1997). "Soil enzyme activities as integrative indicators of soil health," in *Biological Indicators of Soil Health*, eds C. E. Pankhurst, B. M. Doube, and V. V. S. R. Gupta (Wellingford: CABI), 121–156.

Dick, R. P., Breakwell, D., and Turco, R. (1996). "Soil enzyme activities and biodiversity measurements as integrating biological indicators," in *Handbook of Methods for Soil Assessment of Soil Quality*, eds J. W. Doran and A. J. Jones (Madison, WI: Soil Science Society of America), 247–272.

Dick, R. P., Rasmussen, P. E., and Kerle, E. A. (1988). Influence of long-term residue management on soil enzyme activities in relation to soil chemical properties of a wheat-fallow system. *Biol. Fertil. Soils* 6, 159–164. doi: 10.1007/BF00257667

Dinkelaker, B., and Marschner, H. (1992). In vivo demonstration of acid phosphatase activity in the rhizosphere of soil grown plants. *Plant Soil* 144, 199–205. doi: 10.1007/BF00012876

Doran, J. W., and Parkin, T. B. (1994). "Defining and assessing soil quality," in *Defining Soil Quality for A Sustainable Environment*, eds J. W. Doran, D. C. Coleman, D. F. Bezdicek, and B. A. Stewart (Madison, WI: Soil Science Society of America Special Publication), 3–21.

Fließbach, A., and Mäder, P. (2000). Microbial biomass and size density fractions differ between soils of organic and conventional agricultural systems. *Soil Biol. Biochem.* 32, 757–768. doi: 10.1016/S0038-0717(99)00197-2

Forster, D., Andres, C., Verma, R., Zundel, C., Messmer, M. M., and Mäder, P. (2013). Yield and economic performance of organic and conventional cotton-based farming systems - Results from a field trial in India. *PLoS ONE* 8:e81039. doi: 10.1371/journal.pone.0081039

George, T. S., Richardson, A. E., and Simpson, R. J. (2005). Behaviour of plant-derived 621 extracellular phytase upon addition to soil. *Soil Biol. Biochem.* 37, 977–988. doi: 10.1016/j.soilbio.2004.10.016

Gichangi, E. M., Mnkeni, P. N. S., and Brookes, P. C. (2009). Effects of goat manure and inorganic phosphate addition on soil inorganic and microbial biomass phosphorus fractions under laboratory incubation conditions. *Soil Sci. Plant Nutr.* 55, 764–771. doi: 10.1111/j.1747-0765.2009.00415.x

Goupy, J., and Creighton, L. (2007). *Introduction to Design of Experiments with JMP Examples*, 3rd Edn. Cary, NC: SAS Institute Inc.

Gregory, P. J. (2006). *Plant Roots: Growth, Activity and Interactions with Soils*. Oxford: Black-well publishing Ltd., 216. doi: 10.1002/9780470995563

Guan, G., Tu, S., Li, H., Yang, J., Zhang, J., Wen, S., et al. (2012). Phosphorus fertilization modes affect crop yield, nutrient uptake, and soil biological properties in the rice–wheat cropping system. *Soil Sci. Soc. Am. J.* 77, 166–172. doi: 10.2136/sssaj2011.0324

Harvey, P. R., Warren, R. A., and Wakelin, S. (2009). Potential to improve root access to phosphorus: the role of non-symbiotic microbial inoculants in the rhizosphere. *Crop Pasture Sci.* 60, 144–151. doi: 10.1071/CP08084

Hernández, T., Chocano, C., Moreno, J. L., and Garcia, C. (2016). Use of compost as an alternative to conventional inorganic fertilizer in intensive lettuce (*Lactuca sativa* L.) crops- Effects on soil and plant. *Soil Tillage Res.* 160, 14–22. doi: 10.1016/j.still.2016.02.005

Hinsinger, P. (2001). Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* 237, 173–195. doi: 10.1023/A:1013351617532

IFOAM (2006). *The IFOAM Norms for Organic Production and Processing, Version 2005*. Bonn: Die Deutsche Bibliothek.

Iyamuremye, F., and Dick, R. P. (1996). Organic amendments and phosphorus sorption by soils. *Adv. Agron.* 56, 139–185. doi: 10.1016/S0065-2113(08)60181-9

Jimenez, M. P., Horra, A. M., Pruzzo, L., and Palma, R. M. (2002). Soil quality: a new index based on microbiological and biochemical parameter. *Biol. Fertil. Soils* 35, 302–306. doi: 10.1007/s00374-002-0450-z

Joergensen, R. G., and Mueller, T. (1996). The fumigation-extraction method to estimate soil microbial biomass: calibration of the kEC value. *Soil Biol. Biochem.* 28, 33–37. doi: 10.1016/0038-0717(95)00101-8

Johnston, A. E., Poulton, P. R., Fixen, P. E., and Curtin, D. (2014). Phosphorus: its efficient use in agriculture. *Adv. Agron.* 123, 177–228. doi: 10.1016/B978-0-12-420225-2.00005-4

Kandeler, E., Stemmer, M., and Klimanek, E. M. (1999). Response of soil microbial biomass, urease and xylanase within particle fraction to long-term management. *Soil Biol. Biochem.* 31, 261–273. doi: 10.1016/S0038-0717(98)00115-1

Khan, K. S., and Joergensen, R. G. (2009). Changes in microbial biomass and P fractions in biogenic household waste compost amended with inorganic P fertilizers. *Biores. Technol.* 100, 303–309. doi: 10.1016/j.biortech.2008.06.002

Klein, D. A., Loh, T. C., and Goulding, R. L. (1971). A rapid procedure to evaluate dehydrogenase activity of soils low in organic matter. *Soil Biol. Biochem.* 3, 385–387. doi: 10.1016/0038-0717(71)90049-6

Kong, L., Wang, Y. B., Zhao, L. N., and Chen, Z. H. (2009). Enzyme and root activities in surface-flow constructed wetlands. *Chemosphere* 76, 601–608. doi: 10.1016/j.chemosphere.2009.04.056

Kumar, A., Suri, V. K., and Choudhary, A. K. (2014). Influence of inorganic phosphorus, VAM fungi, and irrigation regimes on crop productivity and phosphorus transformations in okra (*Abelmoschus esculentus* L.)–Pea (*Pisum sativum* L.) cropping system in an acid alfisol. *Commun. Soil Sci. Plant Anal.* 45, 953–967. doi: 10.1080/00103624.2013.874025

Lagomarsino, A., Moscatellil, M. C., Di Tizio, A., Mancinelli, R., Greglo, S., and Marinaril, S. (2009). Soil biochemical indicators as a tool to assess the short-term impact of agricultural management on changes in organic C in a Mediterranean environment. *Ecol. Indic.* 9, 518–527. doi: 10.1016/j.ecolind.2008.07.003

Li, X. H., Han, X. Z., Li, H. B., Song, C., Yan, J., and Liang, Y. (2012). Soil chemical and biological properties affected by 21-year application of composted manure with chemical fertilisers in a Chinese mollisol. *Can. J. Soil Sci.* 92, 419–428. doi: 10.4141/cjss2010-046

Liang, Y., Nikolic, M., Peng, Y., Chen, W., and Jiang, Y. (2005). Organic manure stimulates biological activity and barley growth in soil subject to secondary salinization. *Soil Biol. Biochem.* 37, 1185–1195. doi: 10.1016/j.soilbio.2004.11.017

Lynch, J. P., and Brown, K. M. (2008). "Root strategies for phosphorus acquisition," in *The Ecophysiology of Plant-Phosphorus Interactions*. eds P. J. White and J. P. Hammond (Dordrecht: Springer), 83–116. doi: 10.1007/978-1-4020-8435-5_5

Mäder, P., Fliessbach, A., Dubois, D., Gunst, L., Fried, P., and Niggli, U. (2002). Soil fertility and 529 biodiversity in organic farming. *Science* 296, 1694–1697. doi: 10.1126/science.1071148

Malik, M. A., Khan, K. S., Marshchner, P., and Ali, S. (2013). Organic amendments differ in their effect on microbial biomass and activity and on P pools in alkaline soils. *Biol. Fertil. Soils* 49, 415–425. doi: 10.1007/s00374-012-0738-6

Malik, M. A., Marschner, P., and Khan, K. S. (2012). Addition of organic and inorganic P sources to soil- effects on P pools and microorganisms. *Soil Biol. Biochem.* 49, 106–113. doi: 10.1016/j.soilbio.2012.02.013

Mandal, A., Patra, A. K., Singh, D., Swarup, A., and Masto, R. E. (2007). Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during criop development stages. *Bioresour. Technol.* 98, 3585–3592. doi: 10.1016/j.biortech.2006.11.027

Marinari, S., Mancinelli, R., Campiglia, E., and Grego, S. (2006). Chemical and biological indicators of soil quality in organic and conventional farming systems in Central Italy. *Ecol. Indic.* 6, 701–711. doi: 10.1016/j.ecolind.2005.08.029

Masto, R. E., Ansari, M. A., George, J., Selvi, V. A., and Ram, L. C. (2013). Co-application of biochar and lignite fly ash on soil nutrients and biological parameters at different crop growth stages of *Zea mays*. *Ecol. Eng.* 58, 314–322. doi: 10.1016/j.ecoleng.2013.07.011

Mikha, M. M., and Rice, C. W. (2004). Tillage and manure effect on soil and aggregate associated carbon and nitrogen. *Soil Sci. Soc. Am. J.* 68, 809–816. doi: 10.2136/sssaj2004.8090

Nannipieri, P. (1994). "The potential use of soil enzymes as indicators of productivity, sustainability and pollution," in *Soil Biota: Management in Sustainable Farming Systems*, eds C. E. Pankhurst, B. M. Doube, V. V. S. R. Gupta, and P. R. Grace (East Melbourne, VIC: CSIRO), 238–244.

Nayak, D. R., Babu, Y. J., and Adhya, T. K. (2007). Long-term application of compost influences microbial biomass and enzyme activities in a tropical Aeric Endoaquept planted to rice under flooded condition. *Soil Biol. Biochem.* 39, 1897–1906. doi: 10.1016/j.soilbio.2007.02.003

Ndiaye, E. L., Sandeno, J. M., Mcgrath, D., and Dick, R. P. (2000). Integrative biological indicators for detecting changes in soil quality. *Am. J. Alternative Agric.* 15, 26–36. doi: 10.1017/S0889189300008432

Olsen, S. R., Cole, C. V., Watanabe, F. S., and Dean, L. A. (1954). Estimation of available P in soils by extraction with sodium bicarbonates. *Circular* 939, 1–19.

Opala, P. A., Okalebo, J. R., and Othieno, C. O. (2012). Effects of organic and inorganic materials on soil acidity and phosphorus availability in a soil incubation study. *ISRN Agron.* 2012:597216. doi: 10.5402/2012/597216

Ramesh, A., Sharma, S. K., Joshi, O. P., and Khan, I. R. (2011). Phytase, phosphatase activity and P-nutrition of soybean as influenced by inoculation of *Bacillus*. *Indian J. Microbiol.* 51, 94–99. doi: 10.1007/s12088-011-0104-7

Ramesh, A., Sharma, S. K., Yadav, N. P., and Joshi, O. P. (2014). Phosphorus mobilization from native soil P-pool upon inoculation with phytate-mineralizing and phosphate-solubilizing *Bacillus aryabhattai* isolates for improved P-acquisition and growth of soybean and wheat crops in microcosm conditions. *Agric. Res.* 3, 118–127. doi: 10.1007/s40003-014-0105-y

Rao, A. V., and Tarafdar, J. C. (1992). Seasonal changes in available phosphorus and different enzyme activities in arid soils. *Ann. Arid Zone.* 31, 185–189.

Riar, A., and Coventry, D. (2012). "Nitrogen use as a component of sustainable crop systems," in *Agricultural Sustainability: Progress and Prospects in Crop Research*, eds G. S. Bhullar and N. K. Bhullar (London: Academic Press), 63–76.

Richardson, A., Lynch, J., Ryan, P., Delhaize, E., Smith, F., Smith, S., et al. (2011). Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant Soil* 349, 121–156. doi: 10.2527/jas.53804

Richardson, A. E. (1994). "Soil microorganisms and phosphorus availability," in *Soil Biota Management in Sustainable Farming Systems*, eds C. E. Pankhurst, B. M. Doubeand, and V. V. S. Gupta (Melbourne, VIC: CSIRO Publishing), 50–62.

Sánchez, P. A. (2010). Tripling crop yields in tropical Africa. *Nat. Geosci.* 3, 299–300. doi: 10.1098/rstb.2015.0316

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., et al. (2011). Phosphorus dynamics: from soil to plant. *Plant Physiol.* 156, 997–1005. doi: 10.1104/pp.111.175232

Shi, Y., Ziadi, N., Messiga, A. J., Lalande, R., and Hu, Z. (2013). Changes in soil phosphorus fractions for a long-term corn-soybean rotation with tillage and phosphorus fertilization. *Soil Sci. Soc. Am. J.* 77, 1402–1412. doi: 10.2136/sssaj2012.0427

Singh, M., Reddy, K. S., Singh, V. P., and Rupa, T. R. (2007). Phosphorus availability to rice (*Oriza sativa* L.)-wheat (*Triticum estivum* L.) in a Vertisol after eight years of inorganic and organic fertilizer additions. *Bioresour. Technol.* 98, 1474–1481. doi: 10.1016/j.biortech.2006.02.045

Sinsabaugh, R. L., Klug, M. J., Collins, H. P., Yeager, P. E., and Petersen, S. O. (1999). "Characterizing soil microbial communities," in *Standard Soil Methods for Long Term Ecological Research*, eds G. P. Robertson, C. S. Bledsoe, D. C. Coleman, and P. Sollins (New York: Oxford University Press), 318–348.

Sinsabaugh, R. S. (1994). Enzymic analysis of microbial pattern and process. *Biol. Fertil. Soils* 17, 69–74. doi: 10.1007/BF00418675

Stott, D. E., Andrews, S. S., Liebig, M. A., Wienhold, B. J., and Karlen, D. L. (2010). Evaluation of β-glucosidase activity as a soil quality indicator for the soil management assessment framework. *Soil Sci. Soc. Am. J.* 74, 107–119. doi: 10.2136/sssaj2009.0029

Syers, J. K., Johnston, A. E., and Curtin, D. (2008). *Efficiency of Soil and Fertilizer Phosphorus Use: Reconciling Changing Concepts of Soil Phosphorus Behaviour with Agronomic Information.* Rome: FAO.

Tabatabai, M. A. (1994). "Soil enzymes," in *Methods of Soil Analysis: Microbiological and Biochemical Properties*, eds R. W. Weaver, J. S. Angle, and P. S. Bottomley (Madison, WI: Soil Science Society of America), 775–833.

Tabatabai, M. A., and Bremner, J. M. (1969). Use of p-nitrophenylphosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* 1, 301–307. doi: 10.1186/1756-0500-7-221

Tamilselvi, S. M., Chinnadurai, C., Hamuruga, K., Arulmozhiselvan, K., and Balachandran, D. (2015). Effect of long-term nutrient management on biological and biochemical properties of semi-arid tropical Alfisol during maize crop development stages. *Ecol. Indic.* 48, 76–87. doi: 10.1016/j.ecolind.2014.08.001

Tang, J., Leung, A., Leung, C., and Lim, B. L. (2006). Hydrolysis of precipitated phytate by three distinct families of phytases. *Soil Biol. Biochem.* 38, 1316–1324. doi: 10.1016/j.soilbio.2005.08.021

Tarafdar, J. C., and Chhonkar, P. K. (1979). Phosphatase production by microorganisms isolated from diverse types of soils. *Zentralbl. Bacteriol.* 134, 119–124. doi: 10.1016/S0323-6056(79)80037-3

Tarafdar, J. C., and Claassen, N. (1988). Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. *Biol. Fertil. Soils* 5, 308–312. doi: 10.1007/BF00262137

Tarafdar, J. C., Rao, A. V., and Bala, K. (1988). Production of phosphatates by fungi isolated from desert soils. *Folia Microbiol.* 33, 453–457. doi: 10.1007/BF02925770

Tejada, M., Hernandez, M. T., and Garcia, C. (2006). Application of two organic amendments on soil restoration: effects on soil biological properties. *J. Environ. Qual.* 35, 1010–1017. doi: 10.2134/jeq2005.0460

Tu, C., Louws, F. J., Creamer, N. G., Mueller, J. P., Brownie, C., Fager, K., et al. (2006). Responses of soil microbial biomass and N availability to transition strategies from conventional to organic farming systems. *Agric. Ecosyst. Environ.* 113, 206–215. doi: 10.1016/j.agee.2005.09.013

Turner, B. J., McKelvie, I. D., and Haygarth, P. M. (2002). Characterisation of water extractable soil organic phosphorus by phosphatase hydrolysis. *Soil Biol. Biochem.* 34, 27–35. doi: 10.1016/S0038-0717(01)00144-4

Turner, B. L., Newman, S., Cheesman, A. W., and Reddy, K. R. (2007). Sample pretreatment and phosphorus speciation in wetland soils. *Soil Sci. Soc. Am. J.* 71, 1538–1546. doi: 10.2136/sssaj2007.0017

Vance, E. D., Brookes, P. C., and Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass. *Soil Biol. Biochem.* 19, 703–707. doi: 10.1016/0038-0717(87)90052-6

Watanabe, F. S., and Olsen, S. R. (1965). Test of an ascorbic acid method for determining phosphorus in water and NaHCO3 extracts from soil. *Soil Sci. Soc. Am. J. Proc.* 29, 677–678. doi: 10.2136/sssaj1965.03615995002900060025x

Whalen, J. K., Chang, C., Clayton, G. W., and Carefoot, J. P. (2000). Cattle manure amendments can increase the pH of acid soils. *Soil Sci. Soc. Am. J.* 64, 962–966. doi: 10.2136/sssaj2000.643962x

Yan, F., Schubert, S., and Mengel, K. (1996). Soil pH changes during legume growth and application of plant material. *Biol. Fertil. Soils* 23, 236–242. doi: 10.1007/BF00335950

Zhang, F., Shen, J., Zhang, J., Zuo, Y., Li, L., and Chen, X. (2010). Chapter one-rhizosphere processes and management for improving nutrient use efficiency and crop productivity: implications for China. *Adv. Agron.* 107, 1–32. doi: 10.1016/S0065-2113(10)07001-X

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Plant Science

PERSPECTIVE
published: 11 December 2017
doi: 10.3389/fpls.2017.02051

Biochar in the Agroecosystem–Climate-Change–Sustainability Nexus

Vimala D. Nair[1]*, P. K. Ramachandran Nair[2], Biswanath Dari[1], Andressa M. Freitas[1], Nilovna Chatterjee[2] and Felipe M. Pinheiro[2]

[1] Soil and Water Sciences Department, University of Florida, Gainesville, FL, United States, [2] School of Forest Resources and Conservation, University of Florida, Gainesville, FL, United States

Interest in the use of biochar in agriculture has increased exponentially during the past decade. Biochar, when applied to soils is reported to enhance soil carbon sequestration and provide other soil productivity benefits such as reduction of bulk density, enhancement of water-holding capacity and nutrient retention, stabilization of soil organic matter, improvement of microbial activities, and heavy-metal sequestration. Furthermore, biochar application could enhance phosphorus availability in highly weathered tropical soils. Converting the locally available feedstocks and farm wastes to biochar could be important under smallholder farming systems as well, and biochar use may have applications in tree nursery production and specialty-crop management. Thus, biochar can contribute substantially to sustainable agriculture. While these benefits and opportunities look attractive, several problems, and bottlenecks remain to be addressed before widespread production and use of biochar becomes popular. The current state of knowledge is based largely on limited small-scale studies under laboratory and greenhouse conditions. Properties of biochar vary with both the feedstock from which it is produced and the method of production. The availability of feedstock as well as the economic merits, energy needs, and environmental risks—if any—of its large-scale production and use remain to be investigated. Nevertheless, available indications suggest that biochar could play a significant role in facing the challenges posed by climate change and threats to agroecosystem sustainability.

Keywords: feedstocks, highly weathered tropical soil, low-input agriculture, manure, nutrient retention, phosphorus availability, plant biomass

OPEN ACCESS

Edited by:
José Manuel Mirás-Avalos, Universidade de Santiago de Compostela, Spain

Reviewed by:
Mukhtar Ahmed, Pir Mehr Ali Shah Arid Agriculture University, Pakistan
Liming Ye, Ghent University, Belgium
Maria Luz Cayuela, Centro de Edafología y Biología Aplicada del Segura (CSIC), Spain

***Correspondence:**
Vimala D. Nair
vdn@ufl.edu

Specialty section:
This article was submitted to Agroecology and Land Use Systems, a section of the journal Frontiers in Plant Science

Received: *12 July 2017*
Accepted: *15 November 2017*
Published: *11 December 2017*

Citation:
Nair VD, Nair PKR, Dari B, Freitas AM, Chatterjee N and Pinheiro FM (2017) Biochar in the Agroecosystem–Climate-Change–Sustainability Nexus. Front. Plant Sci. 8:2051. doi: 10.3389/fpls.2017.02051

INTRODUCTION

Agroecosystems the world over are under severe stress. Faced with the challenge of feeding the burgeoning population and meeting the ever-growing demands for fiber and other natural products, agricultural and forestry production systems have become highly dependent on chemical products and technological inputs (for example, Mueller et al., 2012). While the resultant production increases have helped eradicate hunger in many parts of the world, the accompanying ecosystem degradation on a massive scale has raised major concerns (Nair P. K. R., 2014). Consequently, farming practices and technologies that can increase and sustain production without ruining the ecosystem were promoted as an approach to addressing these concerns. Thus, numerous terms and rallying themes became prominent in the global land-use arena during the past few decades, such as (in alphabetical order), agroecology, agroforestry, climate-smart agriculture, conservation agriculture, organic agriculture, permaculture, sustainable intensification, and so on. Almost all of them share the objective of minimizing external inputs by building on the efficient use of locally available resources. This has led to focusing attention on some naturally occurring materials as well as products that can be relatively easily assembled from natural resources to substitute or complement the use of synthetic products. Biochar is one such product that has become quite prominent in the recent past. This paper presents a synthesis and evaluation of the current level of knowledge on biochar and its potential role in agroecosystem management in the climate–change–sustainability context.

PROPERTIES OF BIOCHAR: THE CURRENT STATE OF KNOWLEDGE

The International Biochar Initiative (IBI) describes biochar as *"a solid material obtained from the carbonisation of biomass"* (http://www.biochar-international.org/) which occurs when biomass (such as wood, manure, or crop residues) is heated in a closed container with little or no air (Lehmann and Joseph, 2009). Consequent to the realization of the potential role of biochar, there has been a veritable explosion of interest in biochar in the scientific community. Several materials are reported to have been used as biochar feedstock in different parts of the world for improving soil fertility and plant nutrition. A summary of the available scientific reports on biochar, especially those during the past 5 years, focused on its properties and role in plant nutrition and soil management is presented in **Table 1**.

Biochar as a Source of Plant Nutrients

Recent research has showed that elemental composition of a feedstock is not an indication of plant-nutrient availability in the biochar made out of that feedstock. Freitas et al. (2016) found that available P (Mehlich 3) in biochar made from different feedstocks was not at all proportional to the total P concentration of the feedstocks. X-ray diffraction showed that poultry litter biochar contained the mineral whitlockite (a sparingly soluble Ca-P or Ca-Mg-P form), which might be used as a slow-release P fertilizer (Dari et al., 2016). Furthermore, Mehlich 3-extractable K-values in biochar from different feedstocks were also not proportional to the concentration of the nutrients in the "parent" feedstock. Based on these, Freitas et al. (2016) suggested that some nutrient contents of animal-based biochar (e.g., K) would not necessarily be higher than those of plant-based biochars.

The existence of such variability in biochar properties has been well-established (Ippolito et al., 2015), but information on the reasons for such differences is scanty. Pyrolysis is conducted at varying temperatures (**Table 1**), and the temperature is reported to have effect on the quality of biochar produced; a definitive relationship between the two, however, has not been established. Other processing differences could lead to different biochar properties. Thus, it could well be that biochars prepared from the same feedstock could have different characteristics depending on pyrolysis temperature and other conditions.

Biochar + compost mixtures are becoming popular for improving soil fertility and plant growth (Schulz and Glaser, 2012; Prost et al., 2013), especially when biochar is mixed with biomass before composting. A recent review by Godlewska et al. (2017) has pointed out that the effect of biochar on composting depends on biochar and feedstock properties. Some studies indicate the formation of oxygen-containing functional groups during composting, which leads to increase in nutrient retention (Schulz et al., 2014). This practice allows a higher nutrient retention in the biomass, adding to the value of the final product. As concluded by Wu et al. (2017) in another recent review, biochar and composting could alter the physico-chemical properties of both materials. The combination of biochar with compost seems to be a promising source of amendment and an interesting alternative to inorganic fertilizer.

RELATIONSHIP BETWEEN BIOCHAR AND SOIL PROPERTIES

Soil Nutrient Retention

Nutrient retention/loss risk during biochar application depends not only on the nutrient release potential of the biochar, but also the nutrient retention properties of the soil. Dari et al. (2016) showed that P retention in non-calcareous soils is a property of the soil, independent of the nature of the feedstock. Therefore, the biochar from the same source added at a given rate to different soils could have different effects based on the respective soil properties. As in the case of inorganic P additions, any P released by a given biochar will be retained by the soil as long as the threshold P saturation ratio of the non-calcareous soil is not exceeded (Nair V. D., 2014). For example, when the same amount of biochar is added to a sandy soil and a more clayey soil, the sandy soil will begin to release P faster than the clayey soil. The temperature at which biochar is produced may not have any effect on P release property of the biochar-amended soil (Nair et al., 2016); therefore biochar produced using sophisticated techniques or in simple kilns would likely behave similarly on a given soil type.

Soil Aggregate Formation and Stabilization of Soil Organic Matter

The influence of biochar on soil aggregates and physical stabilization of soil organic matter (SOM) in aggregates has been relatively less studied. Wang et al. (2017) demonstrated that, on addition of biochar, soil aggregation markedly differed between two contrasting soils: while biochar amendment dramatically improved aggregate stability in a fine-textured soil, it had no significant impact on a coarse-textured soil. Biochar also increased C storage in macroaggregates of the fine-textured soil and thereby enhanced the physical protection of SOM in the soil by increasing the proportion of C stored within macroaggregates. On the other hand, Fungo et al. (2017) did not find any effect of biochar addition on soil aggregation in a 2-year study on a tropical Ultisol. These studies suggest the effect of biochar addition on soil aggregation and organic matter stabilization is variable depending on the soil type.

TABLE 1 | Summary of major research results reported on the effect of biochar application on plant nutrition and soil nutrient dynamics.

Study Location	Biochar		Soils characteristics		Crop and Study method	Application rate (Mg ha^{-1}); Biochar pH	Crop yield/growth resp. over control	Nutrient and water retention & availability	Reference
	Feedstocks	**(Pyrolysis Temp, ^{0}C)**	**Description**	**pH**					
China	Wheat straw	350–550	Calcareous loamy and silty clay loam	NA¶	Maize and rice; Field	10, 20, 40 with and without N	Corn: 7-12% yield Rice: 8-14% yield ↑	NA¶	(1)
	Wheat straw Maize straw	300–600 400	Upland Red soils (~Ultisols)	6.7	Rapeseed and potato; Field	0, 2.5, 5, 10, 20, 30 & 40	Rapeseed: 36% Potato: 54% yield ↑	soil water stable aggregate soil organic carbon total N and C:N ratio ↑	(2)
	Maize straw Wheat straw	400	Sandy loam and Calcic	NA¶	Rice and maize; Field	2.4	Rice & maize: 6% yield	↑NA¶	(2)
	Wheat straw	350–550	Hydroagric Stagnic Anthrosol	NA¶	Rice; Field	40	18.3% grain yield	↑NA¶	(3)
	Pig manure compost, peanut husk & biosolids	350–450	Entic Hydroagric Anthrosol	NA¶	Rice; Field	0.45	13.5%, 28.1% & 31.4% grain yield	↑NA¶	(4)
	Rice straw	NA¶	Gleyi–Stagnic Anthrosol	NA¶	Rice-wheat; Pot	4.5 & 9	14.8 & 21.3% grain yield	↑	(5)
	Giant reed grass (*Arundo donax*)	300–600	Tropical sandy; 29.2% sand, 13.6% clay	6.02	Maize; Greenhouse column	0, 1, 2, 5% (w/w)	Growth↑	Reduce in NH_4^+-N, Increase in WHC, improve in N bioavailability	(6)
Japan	Chicken manure	402–528	Sandy: 47.5% sand, 11.7% clay	7.0	*Brassica rapa;* Field	10 (pH = 10.5)	90% growth↑	25% mineralization of the total N.	(7)
	Wood-based (Japanese cedar and cypress)	300	Sand-dune soils	6.9	Rice; Field	0, 20 & 40 (pH = 9.8)	Crop yield↑	20–30% to 50–60% increase in available water content	(8)
	Rice husk	350–400	Haplic Andosols	NA¶	Rice; Pot	0.02, 0.2 & 2 kg m^{-2}	14% straw yield↑	NA¶	(9)
USA New York	Maize Stover	600	Kendaia silt loam and Lima loam.	7.36	Maize; Field	0, 1, 3, 12, and 30; + 108 kg N ha^{-1} (pH = 10.0)	No effects on yield	No improvement in crop N use efficiency; N uptake did not change; increased N retention	(10)
Florida	Peanut hull & Brazilian pepperwood	600	Sandy: sand: 94%, clay: 3.0%	5.9	Laboratory column	0.1 g char L^{-1} aqueous solution	Crop yield↑	Decrease in nitrate (34%), ammonium (35%) & phosphate (21%) leaching	(11)
Idaho	Hardwood biochar & dairy manure co-application	500	Calcareous; Portnuef soil	8.2	Lab incubation	0%, 1%, 2%, 10% by wt (pH = 6.8)	NA¶	Improve in soil water content; increase in soil NO_3-N	(12)

(Continued)

TABLE 1 | Continued

Study Location	Biochar		Soils characteristics		Crop and Study method	Application rate (Mg ha^{-1}); Biochar pH	Crop yield/growth resp. over control	Nutrient and water retention & availability	Reference
	Feedstocks	**(Pyrolysis Temp, ^{0}C)**	**Description**	**pH**					
Spain	Bamboo wood, Dairy manure, & mixed wood chip	NA[¶]	Sandy to silty clay loam	6.5	Lab incubation	2% (w/w; dry weight)	NA[¶]	Consistent decrease in N_2O emissions by 10–90%	(13)
	Olive-tree prunings	450	Vertisol: 22% sand, 51% clay	8.2	Wheat; Field	2% by weight (pH = 6.6)	Crop yield↑	Increase in available N, P and C	(14)
Germany	Peanut hull	NA[¶]	Sandy	6.0	Quinua; Greenhouse	100 and 200 char (pH = 8.1)	Crop yield	Increase in leaf N; decrease in greenhouse gas emissions; increase in WHC	(15)
	Maize biochar used as hydro-biochar	600	Loamy sand	6.2	Wheat; Pot	0, 4, 12 (pH = 7.7)	Crop yield↓	No effect on N and Ca contents; decrease in plant tissue N	(16)
Denmark	Straw	730	Coarse sandy	6.5	Barley; pot	0, 8, 16, 32, 64 + (208 N+30 P) fertilizer	Yield: 6.0, 22, −12, −28, 10%↓	↑NUE was not prominent	(17)
Australia	Willow wood	550	Tropical Ferralsol	acidic	Maize; Field	0, 10, 25 + compost (co-composting)	10–29% yield	↑Increase in soil N, P, OC & water content	(18)
Bangladesh	Sawdust	300–350	"Alkaline"	8.0	Soybean; Pot	20 (pH = 5.21)	54% yield↑	Increase in available P	(19)
Finland	Spruce chips (Picea abies)	550–600	Boreal loamy sand 83% sand, 2% clay	4.65	Wheat; Field	0, 5, 10, 20, 30 + inorganic fertilizers	No effects on yield	Increase in soluble K & SOC; no effects on other soil nutrients (N, P); increase in plant-available water content	(20)
Indonesia	Bark of *Acacia mangium*	260–360	"Acidic" soil		Maize; Field	37	12% yield↑	NA[¶]	(21)
Philippines	Rice husk (Chimney charring process)	NA[¶]	Anthraquic gleysols Humic nitisols	6.55 4.3	Rice; Field	4.13 kg m^{-2}	Both ↑(16-35%) &↓ in yield	NA[¶]	(22)

BC, biochar; C, Carbon; Ca, calcium; CUE, cation exchange capacity; K, potassium; N, nitrogen; NUE, nitrogen use efficiency; OC, organic carbon; P, phosphorus; WHC, water-holding capacity.

[¶] *NA, not available. The up arrows and down arrows represent, respectively, the increasing and decreasing responses of the parameters by biochar application.*

References: (1) Zhang et al., 2016; (2). Liu et al., 2014; (3) Bian et al., 2014; (4) Qian et al., 2014; (5) Zhao et al., 2014; (6) Zheng et al., 2013; (7) Ishimori et al., 2017; (8) Kameyama et al., 2017; (9) Koyama and Hayashi, 2017; (10) Guerena et al., 2013; (11) Yao et al., 2012; (12) Ippolito et al., 2016; (13) Cayuela et al., 2013; (14) Olmo et al., 2014; (15) Kammann et al., 2011; (16) Reibe et al., 2015; (17) Bruun et al., 2012; (18) Agegnehu et al., 2016; (19) Mete et al., 2015; (20) Tammeorg et al., 2014; (21) Yamato et al., 2006; (22) Haefele et al., 2011.

Soil Physical Properties

Several studies have reported that biochar addition to soils decreases soil bulk density (BD) and increases water-holding capacity (WHC). Increase in WHC following biochar addition is attributed to high surface area and porosity of biochar (Novak et al., 2009; Kinney et al., 2012; Laghari et al., 2016), which contributes to greater water use efficiency and thus plant productivity. Increase in WHC by biochar additions could be particularly pronounced in sandy soils, where the low surface area of their particles and abundance of macro-pores limit the capacity for holding water. Based on studies using pine-sawdust biochar produced under different temperatures, Laghari et al. (2016) suggested that WHC of desert soils could be improved leading to better plant growth through biochar addition.

Soil Microbial Properties

Thies et al. (2015) reviewed the studies on the influence of biochar on soil microbial properties including microbial biomass, enzyme activities, nitrogen mineralization rates, soil respiration, ratio of bacteria to fungi, and soil- borne diseases. Given the variations among different types of biochar, the interaction effects of biochar with various soils and plants under different climatic conditions can be enormously variable. Consequently, there could be corresponding impacts on plant growth and productivity as well as emission of greenhouse gases.

BIOCHAR IN SOIL CARBON SEQUESTRATION AND CLIMATE-CHANGE MITIGATION

Based on the management practice of the ancient civilizations, the idea of sequestering carbon via biochar addition to soil has been of interest to scientists as a means of mitigating global warming through soil C sequestration. So much so, biochar application to agricultural soils is now considered as a soil-based greenhouse mitigation strategy for sustainable environmental management (Paustian et al., 2016). Management practices that could potentially increase C sequestration in biomass and in the soil by using biochar as a nutrient source also have received some research attention. Following an evaluation of the characteristics of 76 biochars from 40 studies, Brassard et al. (2016) reported that biochars with lower N content (C/N ratio >30) were found to be more suitable for mitigation of N_2O emissions from soil, and those produced at higher pyrolysis temperature might have high C sequestration potential.

One of the important attributes of biochar is that carbon in biochar resists decomposition. Lehmann et al. (2006) reported that biochar "can hold carbon in soils for hundreds to thousands of years" as evidenced by the *Terra Preta* soils of the Amazonian region in Northern Brazil (Glaser et al., 2001). A meta-analysis of decomposition and priming effects on biochar stability in soil (Wang et al., 2016) suggested that only a small percentage of biochar C (3%) is bioavailable and that the remaining contributed to long-term stability in soil. The analysis was based on 128 observations of biochar-derived CO_2 from 24 studies with ^{13}C and radioactive ^{14}C isotopes. However, a systematic review by Gurwick et al. (2013) concluded that: "there are not enough data to draw conclusions about how biochar production and application affect whole-system GHS (greenhouse gas) budgets."

Increasing biomass production, whether for increasing food production, energy generation or for reclaiming degraded land, will remove atmospheric CO_2 and could thus be a mitigating strategy for reducing global warming. Moreover, conversion of agriculture and forestry byproducts into biochar could reduce CO_2 and methane emissions from feedstocks during the natural decomposition or burning of the waste material (http://www.biochar-international.org/biochar/carbon). Overall, it seems reasonable to conclude that biochar's effect on climate change mitigation cannot be established as a cause—effect relationship; but there could be advantages in the longer term.

BIOCHAR AND SUSTAINABLE AGRICULTURE

Sustainability is another "all-encompassing" and difficult-to-measure issue, such that the specific role of biochar in the sustainability paradigm is rather nebulous, just as for climate-change mitigation. A meta-analysis on the effect of biochar and plant productivity/nutrient cycling (Biederman and Harpole, 2012) indicated that there was increased aboveground productivity, crop yield, soil microbial biomass, rhizobial nodulation, and plant K tissue concentration. The authors also indicated that pH, N, P, K and total C in the soil increased compared to control conditions. Jeffery et al. (2013) commented that while meta-analyses are powerful tools for obtaining insights from published literature, they rely heavily on input data, a view the authors of this paper share. Additionally, almost all the issues discussed under effect of biochar on soil properties and many more have relevance to the sustainability issue.

LIMITATIONS OF BIOCHAR USE

Based on available data, Mukherjee and Lal (2014) identified several negative aspects of biochar application to soil. These included leaching losses of C and N, contaminant mobility, and several unfavorable physical changes and changes to soil biota. The authors also identified some negative impacts on agronomic yields, and pointed out that effects of biochar applications on gaseous emissions were contradictory. As **Table 1** that summarizes some of the relatively recent literature on the effect of biochar application on plant nutrition and soil nutrient dynamics shows, the majority of the studies reported positive responses, while a few indicated negative ones. It is also likely that some authors may be reluctant to report negative results.

OPPORTUNITIES FOR BIOCHAR USE

Land-Application of Biochar

Besides greenhouse and laboratory experiments, some field studies have been reported on agricultural use of biochar as a nutrient source and soil amendment (**Table 1**). However, as concluded in a recent review by Agegnehu et al. (2017), a substantial and scientifically rigorous body of knowledge based on large-scale field validation of the purported merits of biochar has not yet been generated. Based on a meta-analysis of the effects of biochar-application on crop yields, Jeffery et al. (2015) concluded that: "while biochar has been shown to have promise for increasing crop productivity, we do not have a mechanistic understanding of the interactions behind observed yield increases to provide universally applicable guidance." In another meta-analysis, Jeffery et al. (2017) reported that the extent and cause of the assumed yield benefit of biochar use was controversial, and that the yield benefits were from nutrient-poor, acidic, tropical soils when high-nutrient biochar inputs were added. The authors also cautioned that the lack of uniformity in the available literature on biochar effects on crop yield could impact the statistical rigor of such meta-analyses.

Low Input Agriculture

The opportunities for using biochar in the low-input agricultural systems that are predominant in developing countries are also worth serious consideration. The smallholder family farms are the mainstay of agriculture in the tropical and subtropical regions. According to FAO statistics, there are 562 M of the so-called small farms out of the total 609 M farms globally. The average size of these farms varies widely among societies and regions, and collectively they account for only 1,260 M ha or roughly 25% of the total agricultural area (http://faostat3.fao.org/faostat-gateway/go/to/home/E). Yet, an estimated 2.6 billion people produce more than 70% of the world's food on these family farms. These smallholder farmers depend heavily on indigenous and locally available materials such as farmyard manure, green manure, and crop residues as soil-fertility resources with only limited use of purchased chemical fertilizers. At the same time, large quantities of agricultural byproducts such as cereal straw and husk, bagasse, and tree limbs that are generated from those multi-species smallholder land-use systems are currently ignored and denigrated as "agricultural waste."

Highly weathered tropical soils are inherently poor in soil fertility because of numerous physical, chemical, and microbiological constraints that limit agricultural production. Available results on the beneficial effect of biochar application to soils in terms of better nutrient relations (e.g., improving P availability, and reducing nutrient leaching), improvement of soil aeration and water-holding capacity, and enhanced microbiological activities (e.g., symbiotic N_2 fixation and mycorrhizal associations) suggest the promising role of biochar under these tropical farming systems. Developing appropriate technologies for converting these "waste" products into biochar could go a long way in enhancing crop yields and maintaining soil health. That will be a "win-win" situation in terms of yield increases and waste disposal for smallholder farmers of developing nations.

The multispecies combinations consisting of intimate association of plants of various types and forms including herbs, shrubs, vines, and trees, all in the same production unit, as in agroforestry systems that are common in many parts of the world might be another niche opportunity for biochar technology adaptation. Farm "wastes" of various types become available in relatively large quantities in land-use systems involving frequently harvested tree crops such as palms, coffee (*Coffea* spp), cacao (*Theobroma cacao*), and a variety of other crops. Promising reports are available on the successful conversion of these byproducts and wastes such as coconut (*Cocos nucifera*) husks, shells and sheaths, outer covering of cacao pods, and a variety of other materials to biochar. Obviously, such operations are of limited scale and applicability, but are important, especially in the production of specialty crops and horticultural industry. It will be a worthwhile effort to undertake market surveys and feasibility assessments of such promising endeavors. Indeed, the whole area of socioeconomics of biochar use in low-input agricultural systems deserves serious attention.

Forestry and Specialty Crops

The potential for biochar applications in forestry, horticulture, and specialty crops is another area that has not been explored seriously. Production of healthy and vigorous seedlings/saplings is of utmost importance in forestry, landscaping and environmental horticulture, fruit trees, commercial plantings of rubber (*Hevea brasiliensis*), oil palm (*Elaeis guaneensis*), tree spices, and such other perennial specialty crops. Given the reported benefits of biochar and the relatively small quantity of biochar that is needed for application to nursery beds and pots (as opposed to field application for crops), both commercial and small-scale nurseries and individual owners of any size of land holdings could be benefitted by biochar use. Spot application of biochar in planting pits of trees is yet another, relatively unexplored opportunity. For example, establishing nitrogen-fixing trees (NFT) in agroforestry systems in acid soils is a challenge because most NFTs as well as the symbiotic nitrogen-fixing bacteria (*Rhizobium* spp.) prefer pH above 5.5 and many humid tropical soils have pH lower than that. Spot application of lime in tree-planting pits is a commonly adopted practice in such situations. Given its reported soil-amendment-, pH-moderating-, and other beneficial effects, biochar could possibly be applied to such planting pits alone or in combination with lime. The high water-holding capacity of biochar could be particularly advantageous in arid and semiarid regions.

CONCLUSIONS

Available evidence and indications strongly justify continued research and development efforts in understanding more about the benefits and potentials as well as limitations of biochar and expanding its use in land management. The beneficial role of biochar application on the broader issues of climate-change mitigation and sustainable agriculture can reasonably be assumed based on the available body of knowledge, but it is abysmally weak—almost non-existent—on socioeconomic issues (the "other hand" of sustainability). In order to accomplish the goal of agroecosystem sustainability, it is essential that the two sectors are strengthened and are then properly integrated as presented schematically in **Figure 1**. Rather than presenting a long "wish list" of "things to do," suffice it to say emphatically that

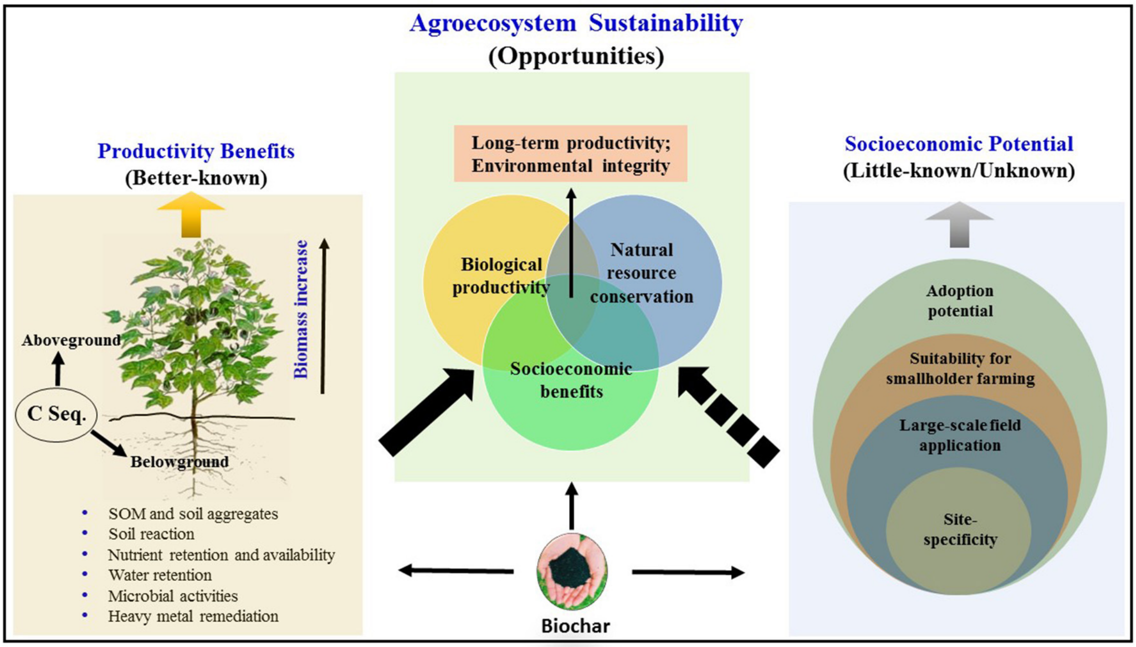

FIGURE 1 | A schematic presentation of the role and potential of biochar in the agroecosystem–climate-change–sustainability nexus. Integration of relatively better-known productivity benefits with the yet-to-be-found solutions to little-known and unknown factors is conceptualized. C = carbon; C seq. = carbon sequestration; SOM = soil organic matter.

while biochar use is not a panacea for solving all the problems of land management, it certainly is an aspect that deserves serious attention in agroecosystem management in the future.

AUTHOR CONTRIBUTIONS

VN: Conceptualized the scope and framework, drafted some sections, and coordinated the efforts. PN: Put together the first draft together with VN and conceptualized **Figure 1**. BD: Developed **Figure 1**, **Table 1**, and assisted in information gathering and discussion. AF, NC, and FP: Collected and collated literature, helped with preparation of **Table 1** and section drafts, and participated in discussion. AF also put the reference list together. All authors have read and approved the submitted manuscript.

FUNDING

Partial salary support of authors of this manuscript by the National Institute of Food and Agriculture, U.S. Department of Agriculture, (NIFA-USDA) under award number 2016-67019-25262 is gratefully acknowledgment. Various biochar analyses in the section on “Biochar as a source of plant nutrients” were completed with support from USDA-Hatch Funds.

REFERENCES

Agegnehu, G., Bass, A. M., Nelson, P. N., and Bird, M. I. (2016). Benefits of biochar, compost and biochar–compost for soil quality, maize yield and greenhouse gas emissions in a tropical agricultural soil. *Sci. Total Environ.* 543, 295–306. doi: 10.1016/j.scitotenv.2015.11.054

Agegnehu, G., Srivastava, A. K., and Bird, M. I. (2017). The role of biochar and biochar-compost in improving soil quality and crop performance: a review. *Appl. Soil Ecol.* 119, 156–170. doi: 10.1016/j.apsoil.2017.06.008

Bian, R., Zhang, A., Li, L., Pan, G., Zheng, J., Zhang, X., et al. (2014). Effect of municipal biowaste biochar on greenhouse gas emissions and metal bioaccumulation in a slightly acidic clay rice paddy. *BioResources* 9, 685–703. doi: 10.15376/biores.9.1.685-703

Biederman, L., and Harpole, W. S. (2012). Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. *GCB Bioenergy* 5, 202–214. doi: 10.1111/gcbb.12037

Brassard, P., Godbout, S., and Raghavan, V. (2016). Soil biochar amendment as a climate change mitigation tool: key parameters and mechanisms involved. *J. Environ. Manag.* 181, 484–497. doi: 10.1016/j.jenvman.2016.06.063

Bruun, E. W., Petersen, C., Strobel, B. W., and Hauggaard-Nielsen, H. (2012). Nitrogen and carbon leaching in repacked sandy soil with added fine particulate biochar. *Soil Sci. Soc. Am. J.* 76, 1142–1148. doi: 10.2136/sssaj2011.0101

Cayuela, M. L., Sanchez-Monedero, M. A., Roig, A., Hanley, K., Enders, A., and Lehmann, J. (2013). Biochar and denitrification in soils: when, how much and why does biochar reduce N_2O emissions? *Sci. Rep.* 3:1732. doi: 10.1038/srep01732

Dari, B., Nair, V. D., Harris, W. G., Nair, P. K. R., Sollenberger, L., and Mylavarapu, R. (2016). Relative influence of soil-vs. biochar properties on soil phosphorus retention. *Geoderma* 280, 82–87. doi: 10.1016/j.geoderma.2016.06.018

Freitas, A. M., Nair, V. D., Harris, W. G., and Mackowiak, C. (2016). “Phosphorus release from various biochar feedstocks: Implications for land management,” *Abstract Retrieved From ASA, CSSA and SSSA International Annual Meetings.* Available online at: https://scisoc.confex.com/crops/2016am/webprogram/Paper101356.html (Accessed July 9, 2017).

Fungo, B., Lehmann, J., Kalbitz, K., Thiongo, M., Okeyo, I., Tenywa, M., et al. (2017). Aggregate size distribution in a biochar-amended tropical Ultisol under conventional hand-hoe tillage. *Soil Tillage Res.* 165, 190–197. doi: 10.1016/j.still.2016.08.012

Glaser, B., Haumaier, L., Guggenberger, G., and Zech, W. (2001). The 'Terra Preta' phenomenon: a model for sustainable agriculture in the humid tropics. *Naturwissenschaften* 88, 37–41. doi: 10.1007/s001140000193

Godlewska, P., Schmidt, H. P., Ok, Y. S., Oleszczuk, P. (2017). Biochar for composting improvement and contaminants reduction. A review. *Bioresour. Technol.* 246, 193–202. doi: 10.1016/j.biortech.2017.07.095

Guerena, D., Lehmann, J., Hanley, K., Enders, A., Hyland, C., and Riha, S. (2013). Nitrogen dynamics following field application of biochar in a temperate North American maize-based production system. *Plant Soil* 365, 239–254. doi: 10.1007/s11104-012-1383-4

Gurwick, N. P., Moore, L. A., Kelly, C., and Elias, P. (2013). A systematic review of biochar research, with a focus on its stability in situ and its promise as a climate mitigation strategy. *PLoS ONE* 8:e75932. doi: 10.1371/journal.pone.0075932

Haefele, S. M., Konboon, Y., Wongboon, W., Amarante, S., Maarifat, A. A., Pfeiffer, E. M., et al. (2011). Effects and fate of biochar from rice residues in rice-based systems. *Field Crops Res.* 121, 430–440. doi: 10.1016/j.fcr.2011.01.014

Ippolito, J. A., Stromberger, M. E., Lentz, R. D., Dungan, R. S. (2016). Hardwood biochar and manure co-application to a calcareous soil. *Chemosphere* 142, 84–91. doi: 10.1016/j.chemosphere.2015.05.039

Ippolito, J. A., and Spokas, K. A., Novak, J. M., and Lentz, R. D., and Cantrell, K. B. (2015). "Biochar elemental composition and factors influencing nutrient retention" in *Biochar for Environmental Management: Science, Technology and Implementation*, *2nd Edn,* eds J. Lehmann and J., Stephen. London; New York, NY: Routledge; Taylor and Francis Group. 137–161.

Ishimori, T., Yoshihiko, T., Hideyuki, S., Amjad, H., Yuzuri, I., Ganesh, N. P., et al. (2017). Low temperature carbonization of chicken manure to char and its effect on growth of *Oryza Sativa*, L. Koshihikari *a*nd Brassica *r*apa Komatsuna. *Euro Mediterr. J. Environ. Integr.* 2:10. doi: 10.1007/s41207-017-0020-2

Jeffery, S., Abalos, D., Prodana, M., Bastos, A. C., van Groenigen, J. W., Hungate, B. A., et al. (2017). Biochar boosts tropical but not temperate crop yields. *Environ. Res. Lett.* 12:053001. doi: 10.1088/1748-9326/aa67bd

Jeffery, S., Bezemer, T. M., Cornelissen, G., Kuyper, T. W., Lehmann, J., Mommer, L., et al. (2015). The way forward in biochar research: targeting trade-offs between the potential wins. *Global Change Biol. Bioenergy* 7, 1–13. doi: 10.1111/gcbb.12132

Jeffery, S., Verheijen, F. G. A., Bastos, A. C., and Van Der Velde, M. (2013). A comment on 'Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis': on the importance of accurate reporting in supporting a fast-moving research field with policy implications. *Global Change Biol. Bioenergy* 6, 176–179. doi: 10.1111/gcbb.12076

Kameyama, K., Iwata, Y., and Miyamoto, T. (2017). Biochar amendment of soils according to their physicochemical properties. *Japn Agric. Res. Q.* 51, 117–127. doi: 10.6090/jarq.51.117

Kammann, C. I., Linsel, S., Gobling, J. W., and Koyro, H. W. (2011). Influence of biochar on drought tolerance of *Chenopodium quinoa* Willd and on soil-plant relations. *Plant Soil* 345, 195–210. doi: 10.1007/s11104-011-0771-5

Kinney, T. J., Masiello, C. A., Dugan, B., Hockaday, W. C., Dean, M. R., Zygourakis, K., et al. (2012). Hydrologic properties of biochars produced at different temperatures. *Biomass Bioenergy* 41, 34–43. doi: 10.1016/j.biombioe.2012.01.033

Koyama, S., and Hayashi, H. (2017). Rice yield and soil carbon dynamics over three years of applying rice husk charcoal to an Andosol paddy field. *Plant Prod. Sci.* 20, 176–182. doi: 10.1080/1343943X.2017.1290506

Laghari, M., Naidu, R., Xiao, B., Hu, Z., Mirjat, M. S., Hu, M., et al. (2016). Recent developments in biochar as an effective tool for agricultural soil management: a review. *J. Sci. Food Agric.* 96, 4840–4849. doi: 10.1002/jsfa.7753

Lehmann, J., Gaunt, J., and Rondon, M. (2006). Bio-char sequestration in terrestrial ecosystems review. *Mitigat. Adapt. Strateg. Glob. Change* 11, 403–427. doi: 10.1007/s11027-005-9006-5

Lehmann, J., and Joseph, S. (2009). Biochar for environmental management : an introduction. *Sci. Technol.* 1, 1–12.

Liu, Z., Chen, X., Jing, Y., Li, Q., Zhang, J., and Huang, Q. (2014). Effects of biochar amendment on rapeseed and sweet potato yields and water stable aggregate in upland red soil. *Catena* 123, 45–51. doi: 10.1016/j.catena.2014.07.005

Mete, F. Z., Mia, S., and Dijkstra, F. A., Abuyusuf, M. D., and Hossain, A. S. M. I. (2015). Synergistic effects of biochar and NPK fertilizer on soybean yield in an alkaline soil. *Pedosphere* 25, 713–719. doi: 10.1016/S1002-0160(15)30052-7

Mueller, N. D., Gerber, J. S., Johnston, M., Ray, D. K., Ramankutty, N., and Foley, J. A. (2012). Closing yield gaps through nutrient and water management. *Nature* 490, 254–257. doi: 10.1038/nature11420

Mukherjee, A., and Lal, R. (2014). The biochar dilemma. *Soil Res.* 52, 217–230. doi: 10.1071/SR13359

Nair, P. K. R. (2014). Grand challenges in agroecology and land-use. *Front. Environ. Sci.* 2:1. doi: 10.3389/fenvs.2014.00001

Nair, V. D. (2014). Soil phosphorus saturation ratio for risk assessment in land use systems. *Front. Environ. Sci.* 2:6. doi: 10.3389/fenvs.2014.00006

Nair, V. D., Freitas, A. M., and Harris, W. G. (2016). "Land application of biochar and phosphorus retention: 'An Inconvenient Truth'," in *Abstract Retrieved from ASA, CSSA and SSSA International Annual Meetings* Available online at: https://scisoc.confex.com/crops/2016am/webprogram/Paper99414.html (Accessed July 9, 2017).

Novak, J. M., Lima, I. M., Xing, B., Gaskin, J. W., Steiner, C., Das, K. C., et al. (2009). Characterization of designer biochar produced at different temperatures and their effects on a loamy sand. *Ann. Environ. Sci.* 3, 195–206.

Olmo, M., Alburquerque, J., Barron, V., del Campillo, M. C., Gallardo, A., Fuentes, M., et al. (2014). Wheat growth and yield responses to biochar addition under Mediterranean climate conditions. *Biol. Fertil. Soils* 50, 1177–1187. doi: 10.1007/s00374-014-0959-y

Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G. P., and Smith, P. (2016). Climate-smart soils. *Nature* 532, 49–57. doi: 10.1038/nature17174

Prost, K., Borchard, N., Siemens, J., Kautz, T., Séquaris, J. M., Möller, A., et al. (2013). Biochar affected by composting with farmyard manure. *J. Environ. Qual.* 42, 164–172. doi: 10.2134/jeq2012.0064

Qian, L., Chen, L., Joseph, S., Pan, G., Li, L., Zheng, J., Zhang, X., et al. (2014). Biochar compound fertilizer as an option to reach high productivity but low carbon intensity in rice agriculture of China. *Carbon Manag*. 5, 145–154. doi: 10.1080/17583004.2014.912866

Reibe, K., Rob, C.-L., and Ellmer, F. (2015). Hydro-/Biochar application to sandy soils: impact on yield components and nutrients of spring wheat in pots. *Arch. Agron. Soil Sci.* 61, 1055–1060. doi: 10.1080/03650340.2014.977786

Schulz, H., Dunst, G., and Glaser, B. (2014). No effect level of co-composted biochar on plant growth and soil properties in a greenhouse experiment. *Agronomy*, 4, 34–51. doi: 10.3390/agronomy4010034

Schulz, H., and Glaser, B. (2012). Effects of biochar compared to organic and inorganic fertilizers on soil quality and plant growth in a greenhouse experiment. *J. Plant Nutr. Soil Sci.* 175, 410–422. doi: 10.1002/jpln.201100143

Tammeorg, P., Simojoki, A., Makela, P., Stoddard, F. L., Alakukku, L., and Helenius, J. (2014). Short-term effects of biochar on soil properties and wheat yield formation with meat bone meal and inorganic fertilizer on a boreal loamy sand. *Agric. Ecosyst. Environ.* 191, 108–116. doi: 10.1016/j.agee.2014.01.007

Thies, J. E., Rillig, M. C., and Graber, E. R. (2015). Biochar effects on the abundance, activity and diversity of the soil biota. *Biochar Environ. Manag. Sci. Technol. Implement*. 327–390.

Wang, D., Fonte, S. J., Parikh, S. J., Six, J., and Scow, K. M. (2017). Biochar additions can enhance soil structure and the physical stabilization of C in aggregates. *Geoderma* 303, 110–117. doi: 10.1016/j.geoderma.2017.05.027

Wang, J., Xiong, Z., and Kuzyakov, Y. (2016). Biochar stability in soil: meta-analysis of decomposition and priming effects. *GCB Bioenergy* 8, 512–523. doi: 10.1111/gcbb.12266

Wu, H., Lai, C., Zeng, G., Liang, J., Chen, J., Xu, J., et al. (2017). The interactions of composting and biochar and their implications for soil amendment and pollution remediation: a review. *Crit. Rev. Biotechnol.* 37, 754–764. doi: 10.1080/07388551.2016.1232696

Yamato, M., Okimori, Y., Wibowo, I. F., Anshori, S., and Ogawa, M. (2006). Effects of the application of charred bark of *Acacia mangium* on the yield of maize, cowpea and peanut, and soil chemical properties in South Sumatra, Indonesia. *Soil Sci. Plant Nutr.* 52, 489–495. doi: 10.1111/j.1747-0765.2006.00065.x

Yao, Y., Gao, B., Zhang, M., Inyang, M., and Zimmerman, A. R. (2012). Effect of biochar amendment on sorption and leaching of nitrate, ammonium, and phosphate in a sandy soil. *Chemosphere* 89, 1467–1471. doi: 10.1016/j.chemosphere.2012.06.002

Zhang, H., Chen, C., Gray, E. M., Boyd, S. E., Yang, H., and Liug, D. (2016). Roles of biochar in improving phosphorus availability in soils: a phosphate adsorbent and a source of available phosphorus. *Geoderma* 276, 1–6. doi: 10.1016/j.geoderma.2016.04.020

Zhao, X., Wang, J., Wang, S., and Xing, G. (2014). Successive straw biochar application as a strategy to sequester carbon and improve fertility: a pot experiment with two rice/wheat rotations in paddy soil. *Plant Soil* 378, 279–294. doi: 10.1007/s11104-014-2025-9

Zheng, H., Zhenyu, W., Xia, D., Stephen, H., and Baoshan, X. (2013). Impacts of adding biochar on nitrogen retention and bioavailability in agricultural soil. *Geoderma* 206, 32–39. doi: 10.1016/j.geoderma.2013.04.018

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Environmental Science

PERSPECTIVE
published: 13 December 2017
doi: 10.3389/fenvs.2017.00088

Managed Multi-strata Tree + Crop Systems: An Agroecological Marvel

*P. K. Ramachandran Nair**

School of Forest Resources and Conservation, University of Florida, Gainesville, FL, United States

Today, when the emphasis on single-species production systems that is cardinal to agricultural and forestry programs the world over has resulted in serious ecosystem imbalances, the virtues of the time-tested practice of growing different species together as in managed Multi-strata Tree + Crop (MTC) systems deserve serious attention. The coconut-palm-based multispecies systems in tropical homegardens and shaded perennial systems are just two such systems. A fundamental ecological principle of these systems is niche complementarity, which implies that systems that are structurally and functionally more complex than crop- or tree monocultures result in greater efficiency of resource (nutrients, light, and water) capture and utilization. Others include spatial and temporal heterogeneity, perennialism, and structural and functional diversity. Unexplored or under-exploited areas of benefits of MTC systems include their ecosystem services such as carbon storage, climate regulation, and biodiversity conservation. These multispecies integrated systems indeed represent an agroecological marvel, the principles of which could be utilized in the design of sustainable as well as productive agroecosystems. Environmental and ecological specificity of MTC systems, however, is a unique feature that restricts their comparison with other land-use systems and extrapolation of the management features used in one location to another.

Keywords: agroforestry, biodiversity conservation, carbon sequestration, specialty crops, tropical homegardens, shaded perennial systems

Edited by:
José Manuel Mirás-Avalos,
Universidade de Santiago de Compostela, Spain

Reviewed by:
Stephen J. Ventura,
University of Wisconsin-Madison, United States
Elizabeth Anne Meier,
Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia

Correspondence:
P. K. Ramachandran Nair
pknair@ufl.edu

Specialty section:
This article was submitted to Agroecology and Land Use Systems, a section of the journal Frontiers in Environmental Science

Received: *03 October 2017*
Accepted: *27 November 2017*
Published: *13 December 2017*

Citation:
Nair PKR (2017) Managed Multi-strata Tree + Crop Systems: An Agroecological Marvel. Front. Environ. Sci. 5:88. doi: 10.3389/fenvs.2017.00088

INTRODUCTION

Some agricultural historians trace back the technological innovations in agriculture to Jethro Tull's invention of the seed drill in 1701. Others consider the scientific investigations on the use of fertilizers that began at the Rothamsted Experimental Station in England in 1843 as the true beginning of technological agriculture. Nevertheless, the dramatic increase in global agricultural production is a phenomenon of the second half the twentieth century. Out of the nearly 200% increase in grain production during that period, only about 30% was the result of increases in area under cultivation; the remaining was made possible by increases in yield per unit area through technology-based agricultural intensification, the so-called Green Revolution (Borlaug, 2007).

These accomplishments have indeed been remarkable. Agricultural intensification, however, is reported to have caused or exacerbated several environmental problems including accelerated soil erosion and degradation, water-quality decline and lowering of water tables, greenhouse-gas build-up and climate change, and biodiversity decline (Mueller et al., 2012). The society at large had to pay a huge overall "price" for reaping the benefits, and yet the benefits were beyond the reach of the vast majority of poor farmers. Moreover, it became infeasible to sustain these benefits in the long run (Pingali, 2012). Furthermore, disruption of intergenerational equity resulting from excessive use of finite resources beyond the regenerative capabilities of nature might deprive the future generations of their ability to access their rightful share of natural capital (Daily and Ehrlich, 1996; Costanza et al., 1997). Today, the importance of conserving the natural resource capital of soil, water, air, and biodiversity is also being recognized while maintaining the main focus on enhancing production of preferred commodities. All these activities are rooted in the notion that modern agricultural and forestry production systems have to be in single-species stands. They entail line planting of plants of uniform age, and if possible genetic make-up, at specified spacing between rows and plants within the rows and monotonously uniform fields. On the other hand, such artificially created landscapes are not found in nature. In the drive for maximizing yield and profit, the age-old farming systems involving plant associations of crops and trees of various forms have been ignored.

The ecosystem imbalance caused by the over-emphasis on single-species production systems is a very complex issue. We certainly need to increase land productivity to meet the growing demands of food and fiber, for which use of non-renewable inputs is considered essential. At the same time we also need to reduce the use of these inputs for the sake of environment and ecology. In the search for such land-use systems, the multi-species tree-based farming systems, based on the age-old practice of growing different species together, deserve serious attention. Although they are not major food-producing systems, there are important lessons to be learned from these agroecosystems that maintain their ecological integrity in spite of being continuously impacted by human exploitation of the wide variety of products and services. This paper assesses the unique characteristics of such managed Multi-strata Tree + Crop (MTC) systems, explores the ecological foundations upon which they are grounded, and argues for finding ways to extrapolate those principles to other land-use systems.

MANAGED MULTI-STRATA TREE + CROP SYSTEMS

Integrated MTC systems are found all over the world. Indeed, wherever land is not deliberately brought under single species systems of crops and trees as in agricultural/grazing, horticultural, and forestry operations, the vegetation will consist of multi-species stands. But, managed MTC systems are a predominant land-use feature of warmer parts of the world, and are an important category of agroforestry systems (AFS).Two groups of such systems with unique characteristics that have received some scientific attention are considered here along with their ecosystem characteristics and resource-utilization features.

Coconut-Palm-Based Multispecies Systems and Homegardens

Palms, belonging to the distinctive botanical family Palmae or Arecaceae, are among the most common perennial plants (trees) and are distributed in the tropical and subtropical regions (Johnson, 2011; Smith, 2014). The most widely cultivated among them is the coconut palm (*Cocos nucifera*), one of the earliest domesticated plants; its uses are legion (Purseglove, 1972). Unlike other cultivated palms that are grown mostly in sole stands, the coconut is usually grown in intimate association with other species, making it perhaps the most widely intercropped tree. The palm has been and still is an inseparable part of the socio-cultural heritage and economic wellbeing of the inhabitants of its major growing regions. Because of the high population density and small landholding sizes in such regions, coconuts are grown mostly in smallholder farms of less than 2 ha. Being a single-stemmed perennial with no cambium, the main stem (trunk) of the palm does not increase in girth with age, and its apical crown at the growing tip of the trunk contains 30–40 long leaves at any time and a fairly constant-sized crown with a diameter of about 7 m throughout its adult life from about 10 to 70 years. In a planted stand of palms of same age, this characteristic growth habit allows considerable light penetration to the plantation floor as the palm grows taller with age, allowing growth of other species under or between them. Thus, smallholder farms of coconut consist mostly of palms in association with a variety of other specialty species of all types: herbs, shrubs, vines, and trees (**Figure 1**), all managed as family-farm enterprises.

Numerous reports are available on the extent of intercropping and the types of crops grown in different countries and regions. The species so intercropped consist of food crops including roots and tubers, fruit trees and MPT, medicinal plants, and others that provide multiple products such as food, fuel, fodder, timber, medicine, and such other basic necessities, and help meet the cash requirements of the growers (Kumar, 2011). These integrated farming systems generally outperform the normal or commercial farming systems in all four dimensions of a multifunctional agriculture: food security, environmental functions, economic functions, and social functions (Tipraqsa et al., 2007).

Homegardens, especially in the tropics, present the most intense assemblage species in a managed community of plants. Coconut palms and several other fruit- and nut-producing species and crops are dominant components of such systems in homesteads in different parts of the world, most notably in the highly populated regions of South and Southeast Asia (Kumar and Nair, 2006). Concerns have been raised about the likelihood of labor-intensive homegardens being replaced by commercial

FIGURE 1 | A managed multi-strata tree–crop (MTC) system consisting of a variety of economically useful species (banana, black pepper, clove trees, pineapple, and others), grown in intimate association with coconut palms on the west coast of India. Banana: *Musa* spp, Black pepper: *Piper nigrum,* Clove trees: *Syzygium aromaticum,* Pineapple: *Ananas comosus.*

farming in the wake of socioeconomic and technological changes. Recent studies in Kerala, India, a well-known hotspot of tropical homegardens, however, have found little evidence for such apprehensions (Fox et al., 2017).

The ecological, managerial, and socio-cultural attributes of tropical homegardens can also be found in similar approaches to multispecies system management such as permaculture (Permanent Agriculture: Mollison, 1994), and Forest Farming (Hart, 1993) that is now gaining popularity in the UK (Pilgrim, 2014). Numerous other such integrated systems are practiced around the world; but several of them are seldom known outside their places of existence. Nair et al. (2016) described them as Cinderella AFS that hold enormous promise for the future if they are brought under the realm of modern research. While tracing the history of development of agroforestry, several authors have described how many of the AFS of today have evolved from such indigenous systems around the world (Herzog, 1998; Kumar and Nair, 2006, 2011; Miller and Nair, 2006; Papanastasis et al., 2009).

Shaded Perennial Systems

"Shaded perennial system" is a term that is used in agroforestry literature for managed, vertically stratified plant associations involving shade-tolerant and/or shade-adapted crops under tall-growing trees. The overstory species of these combinations include those that are either deliberately planted as shade trees as in plantations of cacao (*Theobroma cacao*), coffee (*Coffea* spp.), and tea (*Camellia sinensis*). A large number of economic tree/shrub/vine species are grown under such partial-shade conditions in a variety of situations. Excellent examples of such traditional specialty crop associations from the Pacific Islands are described by Elevitch (2006, 2011). Non-traditional species that are getting popularized lately in such systems include a variety of perennial species such as moringa (*Moringa oleifera*) and high-value specialty species such as sandalwood (*Santalum* spp.) (S. Viswanath, personal communication, 2017).

Information on the extent of area under shaded perennial systems is not readily available. Cacao, a native of the Amazon region, is an understory species in its native habitat, and is cultivated almost exclusively under the shade of a variety of trees and banana. As for coffee, the shade vs. sun coffee discussion is as old as the history of coffee cultivation itself. While coffee grown under shade ("shade coffee") is unquestionably superior to "sun coffee" in terms of aroma and taste and fetches much higher price, the area under shade coffee has gradually been declining because of economic reasons: sun-grown coffee cultivated with rather heavy input of chemicals to keep insects, diseases, and weeds under check far out-yield shade coffee. According to the Millennium Ecosystem Assessment (MEA, 2005), shaded perennial AFS render ecosystem services with high value for supporting human livelihoods include carbon storage, regulation of climate, biodiversity conservation, provision of clean water, and maintenance of soil fertility.

Although the two types of systems mentioned above share the multi-strata canopy configuration that is characteristic of all MTCs, structurally and functionally these systems are different. The shaded perennial systems usually contain only two major, usually woody, species whereas the homegardens consist of higher number of plant species of different forms (trees, shrubs, herbs, vines). Another difference is the extent of socio-cultural interplay in the management of these systems. Homegardens are in smallholder family farms of less than a hectare area, managed mostly by family labor with minimal to no use of chemicals and machinery, whereas shaded perennial systems are commercial operations involving hired labor and machinery.

ECOLOGICAL FOUNDATIONS OF MULTI-SPECIES SYSTEMS

Niche Complementarity

One of the ecological foundations of the MTC systems is the Niche Complementarity Hypothesis (Harper, 1977), which states that "a larger array of species in a system leads to a broader spectrum of resource utilization making the system more productive, and leads to better and more efficient use and sharing of resources." This implies that land-use systems that are structurally and functionally more complex than either crop- or tree monocultures result in greater efficiency of resource (nutrients, light, and water) capture and utilization, and greater structural diversity that entails tighter nutrient cycles. As Tilman and Snell-Rood (2014) have stated, "niche differences among species help to explain why large numbers of competing species coexist, and why greater plant diversity leads to greater ecosystem productivity." While the above- and below-ground diversity provides more system stability and resilience at the site-level, the systems provide connectivity with forests and other landscape features at the landscape and watershed levels.

Systems Perspective

A common thread found in the many definitions and descriptions of AFS/MTCs is their multi-faceted nature. Spatial and temporal heterogeneity, perennialism, and the structural and functional diversity are the ecological properties that are fundamental to such systems (Nair et al., 2008). Comparisons are usually made with natural forested or agroecosystems in terms of the extent to which these properties are maintained in AFS. For example, compared with the net primary productivity of 2–6 Mg dry matter (biomass) ha^{-1} $year^{-1}$ (depending on species) for temperate coniferous forest plantations, the multi-strata homegardens and shaded perennial systems of the tropics can have in excess of 15 Mg ha^{-1} $year^{-1}$. The ecological indices for species similarity, diversity, and richness (Sorenson's, Shannon-Wiener, and Margalef, respectively) of multispecies homegardens are similar to those of nearby primary forests (Kumar, 2011). These similarities with natural ecosystems are strong indicators of ecological sustainability.

Ecosystem Services

A major area of relatively unexplored potential of the MTC systems is their ecosystem services. Among the several such services that are mentioned as potential benefits (Minang and Sassen, 2015), carbon sequestration and biodiversity are two that have received some research attention lately. In these systems, a significant part of the nearly 25% of total biomass production that goes into roots will remain in the soil for periods longer than in annual cropping systems. Scientific data accumulated over the past 20 years of our work show increase in soil carbon (C) stock under agroforestry system under different ecological conditions, and a higher percentage of that C in AFS (compared to treeless systems) is in smaller (silt-and-clay) fractions of soil, indicating recalcitrant nature and long-term storage of C. Increase in soil organic carbon stock, which is important from the soil-fertility-improvement as well as environmental-amelioration (carbon sequestration) points of view.

Biodiversity is proving to be one of humanity's best defenses against extreme weather and rising temperatures; protecting it is important for keeping the ecosystems working for us, providing food, absorbing waste, and protecting shorelines (Duffy et al., 2017). The inherently high level of biodiversity of multispecies systems offers several possibilities for arrangement of various tree/shrub/and grass components according to the needs and preferences of farmers. For example, Webb and Kabir (2009) reported, based on an extensive study in Bangladesh, that the ubiquitous homegardens covered more than 12% of the land area and provided the majority of tree-dominated habitats across the country. The authors articulated that homegardens represented the only real opportunity to conserve plant and wildlife populations outside of the beleaguered protected-area system. It remains unclear, however, whether few or many of the species in an ecosystem are needed to sustain the provisioning of ecosystem services. Isbell et al. (2011) showed, based on a study of 17 biodiversity experiments, that although species diversity may appear functionally redundant for one set of environmental conditions, many species are needed to maintain multiple functions at multiple times and places in a changing world.

FUTURE SCENARIOS AND DIRECTIONS

Environmental and ecological specificity of MTC systems is a unique feature that restricts the comparison of systems at different locations and extrapolation from one location to another. This issue needs to be analyzed in the context of current research advances in the broad arena of land-use systems. Admittedly, the Green Revolution is perceived as the most impactful advance in this area during post-World War II era, and has become a standard against which other advances are compared. Although substantial advances have been made on several fronts such as climate-change mitigation and adaptation, and the use of computer modeling and GMOs (genetically modified organisms) to name a few, they pale when compared to the above-referenced "standard."

A case in point is computer modeling. From the perspective of MTC systems, the scenario is rather hazy. Most of the seemingly reliable crop models are limited to single-species systems where the interaction between plants are restricted to resource utilization among same species (Steduto et al., 2009). This is not to ignore or belittle the modeling work on intercropping systems, and on tree-crop interactions including WaNuLCAS (VanNoordwijk and Lusiana, 1998) and the SAFE family of models (Vander Werf et al., 2007; Graves et al., 2011). As Luedeling et al. (2014) and Bayala et al. (2015) have pointed out, the complex nature of arrangement of species within agroforestry systems hinders the progress in their modeling. Research-based knowledge on the specific management for each component while grown in combination with other species, and the scope for development of varieties are two important management-related research priorities. These are equally challenging to both modelers and field-oriented researchers.

The increasing importance being given to largescale computer models and predictions also is noteworthy in this context. Numerous estimates are available on the potential and magnitude of various ecosystem services; for example, global estimations and predictions on C sequestration (Paustian et al., 2016), and global economic valuations of ecosystem services (Kubiszewski et al., 2017). Costanza et al. (2014) estimated that between 1997 and 2011 the global value of these services decreased by an estimated USD 20 trillion/year due to land-use change. Kubiszewski et al. (2017) predicted that under different scenarios, the global value of ecosystem services could decline by $51 trillion/year or increase by USD $30 trillion/year. To what extent such valuations are meaningful, and whether the site-specificity of agroecosystems is factored into such global estimations are unknown. Given the extremely site-specific nature of the MTC systems, studies at the field level should be the starting points for valuing the benefits of their ecosystem services. Furthermore, often they are expressions of interactions involving not only easily measurable biophysical factors but also difficult-to-quantify sociocultural factors. This is particularly true in low-resource farming situations in the tropics where such practices are common.

The accumulated weight of evidence emerging from various activities of similar nature including the consistency of experience across geographical regions supports the conclusion that these integrated MTCs represent an agroecological marvel. Unfortunately such systems have not received deserving research and policy attention. This is primarily because they do not fit into the single-species model of agricultural development paradigms. Serious efforts are needed to learn the principles based upon which these systems have stood the test of time. When properly understood, those principles could be applied for improvement of extensive food-production systems such as intercropping of annual crops (e.g., maize and beans in Africa and Latin America), and the extensive parkland systems of Africa.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

ACKNOWLEDGMENTS

Nilovna Chatterjee helped with literature collection especially related to application of modeling in agricultural systems, and Vimala Nair read through the manuscript.

This work was supported by USDA/NIFA/Mcintire-Stennis Project FLA-FOR-005249, Accession Number 233673; the views expressed are the author's and not of USDA/NIFA.

REFERENCES

Bayala, J., Sanou, J., Teklehaimanot, Z., and Sinclair, F. (2015). "Adaptation of crops to partial shade in mixed cropping systems," in *Tree- Crop Interactions: Agroforestry in a Changing Climate,* eds C. Black, J. Wilson and C. K. Ong (Wallingford: CABI), 309–325.

Borlaug, N. E. (2007). Sixty-two years of fighting hunger: personal recollections. *Euphytica* 157, 287–297. Available online at: http://repository.cimmyt.org/xmlui/handle/10883/1926?show=full

Costanza, R., de Groot, R., Sutton, P. C., van der Ploeg, S., Anderson, S., Kubiszewski, I., et al. (2014). Changes in the global value of ecosystem services.*Glob. Environ. Change* 26, 152–158. doi: 10.1016/j.gloenvcha.2014.04.002

Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., et al. (1997). The value of the world's ecosystem services and natural capital. *Nature* 387, 253–260.

Daily, G. C., and Ehrlich, P. R. (1996). Socioeconomic equity, sustainability, and Earth's carrying capacity. *Ecol. Appl.* 6, 991–1001.

Duffy, J. E., Godwin, C. M., and Cardinale, B. J. (2017). Biodiversity effects in the wild are common and as strong as key drivers of productivity. *Nature* 549, 261–264. doi: 10.1038/nature23886

Elevitch, C. R. (ed.). (2006). *Traditional Trees of Pacific Islands*. Holualoa, HI: Permanent Agriculture Resources.

Elevitch, C. R. (ed.). (2011). *Specialty Crops for Pacific Islands*. Holualoa, HI: Permanent Agriculture Resources.

Fox, T. A., Rhemtulla, J. M., Ramankutty, N., Lesk, C., Coyle, T., and Kunhamu, T. K. (2017). Agricultural land-use changes in Kerala, India: perspectives from above and below the canopy. *Agric. Ecosyst. Environ.* 245, 1–10. doi: 10.1016/j.agee.2017.05.002

Graves, A. R., Burgess, P. J., Liagre, F., Terreaux, J.-P., Borrel, T., Dupraz, C., et al. (2011). Farm-SAFE: the process of developing a plot- and farm-scale model of arable, forestry, and silvoarable economics. *Agroforestry Syst.* 81, 93–108. doi: 10.1007/s10457-010-9363-2

Harper, J. L. (1977). *Population Biology of Plants*. New York, NY: Academic Press.

Hart, R. (1993). *The Forest Garden*. London: The Institute of Social Innovations.

Herzog, F. (1998). Streuobst: a traditional agroforestry system as a model for agroforestry development in temperate Europe. *Agroforestry Syst.* 42, 61–80.

Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W. S., Reich, P. B., et al. (2011). High plant diversity is needed to maintain ecosystem services. *Nature* 477, 199–202. doi: 10.1038/nature10282

Johnson, D. V. (2011). *Tropical Palms. 2010 Revision*. Non-Wood Forest Products 10/Rev. 1. FAO, Rome.

Kubiszewski, I., Costanza, R., Anderson, S., and Sutton, P. (2017). The future value of ecosystem services: global scenarios and national implications. *Ecosyst. Serv.* 26, 289–301. doi: 10.1016/j.ecoser.2017.05.004

Kumar, B. M. (2011). Species richness and aboveground carbon stocks in the homegardens of central Kerala, India. *Agric. Ecosyst. Environ.* 140, 430–440. doi: 10.1016/j.agee.2011.01.006

Kumar, B. M., and Nair, P. K. R. (eds). (2006). *Tropical Homegardens: A Time-Tested Example of Sustainable Agroforestry. Advances in Agroforestry, Vol. 3.* Dordrecht: Springer Science, 390.

Kumar, B. M., and Nair, P. K. R. (eds). (2011). *Carbon Sequestration in Agroforestry Systems: Opportunities and Challenges. Advances in Agroforestry, Vol. 8.* Dordrecht: Springer Science, 307.

Luedeling, E., Huth, N. I., Kindt, R., and König, K. (2014). Agroforestry systems in a changing climate – challenges in projecting future performance. *Curr. Opin. Environ. Sustain.* 6, 1–7. doi: 10.1016/j.cosust.2013.07.013

MEA (2005). *Millennium Ecosystem Assessment: Ecosystems and Human Well-Being: Synthesis*. Washington, DC: Island Press

Miller, R. P., and Nair, P. K. R. (2006). Indigenous agroforestry systems in Amazonia: from prehistory to today. *Agroforestry Syst.* 66, 151–164. doi: 10.1007/s10457-005-6074-1

Minang, P. A., and Sassen, M. (2015). *An Ecosystem Services Based Analysis of Agroforestry Systems*. Nairobi: ICRAF/UNEP

Mollison, B. (1994). *Introduction to Permaculture*. Tyalgum, NSW: Tagari Publications

Mueller, N., Gerber, J., Johnston, M., Ray, D., Ramankutty, N., and Foley, J. (2012). Closing yield gaps through nutrient and water management. *Nature* 490, 254–257. doi: 10.1038/nature11420

Nair, P. K. R., Gordon, A. M., and Mosquera-Losada, M.-R. (2008). "Agroforestry," in *Ecological Engineering, Vol [1] of Encyclopedia of Ecology, 5 Vols,* eds S. E. Jorgensen and B. D. Fath (Oxford: Elsevier), 101–110.

Nair, P. K. R., Viswanath, S., and Lubina, P. A. (2016). *Cinderella* agroforestry systems. *Agroforestry Syst.* 91, 901–917. doi: 10.1007/s10457-016-9966-3

Papanastasis, V. P., Mantzanas, K., Dini-Papanastasi, O., and Ispikoudis, I. (2009). "Traditional agroforestry systems and their evolution in Greece," in *Agroforestry in Europe*, eds A. Rigueiro-Rodríguez, J. McAdam, and M. R. Mosquera-Losada (Dordrecht: Springer), 89–110.

Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G. P., and Smith, P. (2016). Climate-smart soils. *Nature* 532, 49–57. doi: 10.1038/nature17174

Pilgrim, E. S. (2014). *Dining on Forest Food: Lesson from the Tropics*. London: British Ecological Society.

Pingali, P. L. (2012). Green revolution: impacts, limits, and the path ahead. *Proc. Natl. Acad. Sci. U.S.A.* 109, 12302–12308. doi: 10.1073/pnas.0912953109

Purseglove, J. (1972). *Tropical Crops: Monocotyledons*. London: Longman.

Smith, N. J. H. (2014). *Palms and People in the Amazon*. Dordrecht: Springer, 500.

Steduto, P., Hsiao, T. C., Raes, D., and Fereres, E. (2009). Aquacrop-the FAO crop model to simulate yield response to water: I. concepts and underlying principles. *Agron. J.* 101, 426–437. doi: 10.2134/agronj2008.0139s

Tilman, D., and Snell-Rood, E. (2014). Diversity breeds complementarity. *Nature* 515, 44–45. doi: 10.1038/nature13929

Tipraqsa, P., Craswell, E. T., Noble, A. D., and Schmidt-Vogt, D. (2007). Resource integration for multiple benefits: Multifunctionality of integrated farming systems in Northeast Thailand. *Agric. Syst.* 94, 694–703. doi: 10.1016/j.agsy.2007.02.009

Webb, E. L., and Kabir, M. E. (2009). Homegardening for tropical biodiversity conservation. *Conserv. Biol.* 23, 1641–1644. doi: 10.1111/j.1523-1739.2009.01267.x

VanNoordwijk, M., and Lusiana, B. (1998). WaNulCAS, a model ofwater, nutrient and light cap- ture in agroforestry systems. *Agroforestry Syst.* 43, 217–242.

Vander Werf, W., Keesman, K., Burgess, P., Graves, A., Pilbeam, D., Incoll, L. D., et al. (2007). Yield-SAFE: a parameter-sparse, process-based dynamic model for predicting resource capture, growth, and production in agroforestry systems. *Ecol. Eng.* 29, 419–433. doi: 10.1016/j.ecoleng.2006.09.017

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REVIEW
published: 05 December 2017
doi: 10.3389/fpls.2017.02069

The Value of Native Plants and Local Production in an Era of Global Agriculture

Oren Shelef [1]*, Peter J. Weisberg [2] and Frederick D. Provenza [3]

[1] Biology Department, University of Nevada, Reno, Reno, NV, United States, [2] Department of Natural Resources and Environmental Science, University of Nevada, Reno, Reno, NV, United States, [3] Department of Wildland Resources, Utah State University, Logan, UT, United States

For addressing potential food shortages, a fundamental tradeoff exists between investing more resources to increasing productivity of existing crops, as opposed to increasing crop diversity by incorporating more species. We explore ways to use local plants as food resources and the potential to promote food diversity and agricultural resilience. We discuss how use of local plants and the practice of local agriculture can contribute to ongoing adaptability in times of global change. Most food crops are now produced, transported, and consumed long distances from their homelands of origin. At the same time, research and practices are directed primarily at improving the productivity of a small number of existing crops that form the cornerstone of a global food economy, rather than to increasing crop diversity. The result is a loss of agro-biodiversity, leading to a food industry that is more susceptible to abiotic and biotic stressors, and more at risk of catastrophic losses. Humans cultivate only about 150 of an estimated 30,000 edible plant species worldwide, with only 30 plant species comprising the vast majority of our diets. To some extent, these practices explain the food disparity among human populations, where nearly 1 billion people suffer insufficient nutrition and 2 billion people are obese or overweight. Commercial uses of new crops and wild plants of local origin have the potential to diversify global food production and better enable local adaptation to the diverse environments humans inhabit. We discuss the advantages, obstacles, and risks of using local plants. We also describe a case study—the missed opportunity to produce pine nuts commercially in the Western United States. We discuss the potential consequences of using local pine nuts rather than importing them overseas. Finally, we provide a list of edible native plants, and synthesize the state of research concerning the potential and challenges in using them for food production. The goal of our synthesis is to support more local food production using native plants in an ecologically sustainable manner.

Keywords: regenerative agriculture, local food, domestication, plant utilization, *Pinus monophylla, Pinus edulis*

Edited by:
José Manuel Mirás-Avalos, Universidade de Santiago de Compostela, Spain

Reviewed by:
Alexander Ignatov, R&D Center "Phytoengineering" LLS, Russia
Shabir Hussain Wani, Michigan State University, United States

***Correspondence:**
Oren Shelef
milioren4@gmail.com;
orens@unr.edu

Specialty section:
This article was submitted to Agroecology and Land Use Systems, a section of the journal Frontiers in Plant Science

Received: 28 September 2017
Accepted: 20 November 2017
Published: 05 December 2017

Citation:
Shelef O, Weisberg PJ and Provenza FD (2017) The Value of Native Plants and Local Production in an Era of Global Agriculture. Front. Plant Sci. 8:2069. doi: 10.3389/fpls.2017.02069

REGENERATIVE AGRICULTURE IN A GLOBAL ECONOMY

Feeding growing populations with increasing demands for quality, healthy, savory, and attractive food is a vital challenge for humanity. Contemporary agricultural practices have endeavored to do so by improving productivity of a small number of existing crops, rather than by increasing crop diversity. Developing new crops and learning to use wild plants creates the potential to diversify global food production and better enable local adaptation to the diverse and changing environments humans inhabit (Provenza, 2008). Manifestations of global changes—climatic, ecological, behavioral, and technological—emphasize the need to improve food production in ways that reduce negative impacts on the carrying capacities of the ecosystems we rely upon to sustain us. Regenerative-ecological agriculture can restore earth and human health through the five processes that enable and link all life: flow of energy, captured by plants through photosynthesis; soil-mineral cycles that provides nutrients for life; the water cycle essential for life; ecological relationships that create soil-plant-animal communities; and human-land linkages including landscape-genomics and our dialogue with nature (Massy, 2017). As part of those essential linkages, we could also benefit from re-learning to use local plants as sources of healthy food and other products, with attention and concern for environmental issues. Humans have used plants in many ways that include various forms of domestication, gathering, horticulture (Harris and Fuller, 2014), aquaculture and production of secondary products like grazing (livestock, bees) and forestry. While the use of animals for food and other products also has a fundamental role in agriculture, in this review we focus on plant-based agriculture.

Shelef et al. (2018) describe four aspects of sustainable agriculture: land management, resource management, the human interface, and the ecosystem interface. They argue that using native plants as part of local food production can help create more sustainable agriculture. While local food production has attracted much attention recently, use of native plants in local food production has received little attention. Most food crops are produced, transported, and consumed long distances from their location of origin. Moreover, according to the Food and Agriculture Organization of the United Nations (FAO), more than 90% of the calories humans consume come from just 30 plant species (Hammer et al., 2003). We cultivate only about 150 out of an estimated 30,000 edible plant species (Sethi, 2015 and references within). Within these few species, genetic diversity has decreased as the number of marketed varieties has shrunk. For example, out of more than 7000 varieties of apples grown in the United States in the last century, over 6000 varieties have become extinct (Shand, 2000). At the same time, research efforts focus primarily on improving productivity of a few existing crop species, rather than increasing crop diversity. This represents a serious loss of agro-biodiversity and erosion of genetic diversity, leading to a food industry and human populations more susceptible to stressors associated with global environmental change. Sethi (2015) described the potential loss of food diversity in detail and the FAO estimates there has been a 75% reduction in crop diversity globally.

In this review, we discuss the tradeoffs between efforts to improve the productivity of a limited number of crops and efforts to increase crop diversity by recruiting new species and using local species. We describe the concepts of local agriculture and use of native species, elaborating on the ways these concepts are perceived today. Commercial uses of new crops and wild plants have potential, through diversification, to make global food production more sustainable and resilient. We discuss the advantages, obstacles, and risks associated with using local plants. We also provide a case study—the missed opportunity to utilize locally produced pine nuts at large scale in the Western United States. Finally, we provide a list of consumable native plants, and analyze research endeavors to study them.

In the process of using plants over thousands of years humans have influenced plant evolution (Harris and Hillman, 1989). The early days of agriculture began about 10,000 years ago (Zohary et al., 2012), when people used local species and selected for desirable traits for human consumption (Diamond, 2002). Domestication began with the cultivation of wheat in the Fertile Crescent and rapidly spread throughout Europe (Zohary et al., 2012). Once domesticated, many crops expanded rapidly and are now used in areas where they did not originate (Drewnowski and Popkin, 1997). To a large extent, this is the case with the seven most globally used food crops: rice (*Oryza sativa*), wheat (*Triticum aestivum*), soybeans (*Glycine max*), sugarcane (*Saccharum* spp.), tomato (*Solanum lycopersicum)*, maize (*Zea mays*), and potato (*Solanum tuberosum*) (FAO, 2016). In the United States, nearly all of the plants people consume are exotic species, such as corn, rice, wheat, and soybeans (Pimentel et al., 2005). Most research is now devoted to improving existing crops through artificial selection and breeding, agro-technical approaches and genetic modifications (Lemaux, 2009). New crops developed from local species are the exception (Shelef et al., 2016). Intensive agricultural practices developed to increase yield are associated with ecological and environmental costs that include reducing biodiversity, accelerating land degradation, applying fertilizers, contaminating water and spreading pesticides hazardous to human health (Horrigan et al., 2002; Massy, 2017). Future agriculture will have to cope with increasing food demands for greater populations in the face of changing climates, including changes in the frequency and intensity of precipitation, increasing occurrence of droughts (Howden et al., 2007), and increasing use of chemicals (Boxall et al., 2009). Developing new plant varieties for crop production can help mitigate these challenges by increasing the opportunity to match local crop species with changing environmental conditions.

WHAT WE TALK ABOUT WHEN WE TALK ABOUT LOCAL AGRICULTURE

Local agriculture has two facets. One is use of native plant species that often have not been studied or commercialized. The other is food production, which involves a short distance life cycle from field to plate. Shorter cycles between production and consumption reduce carbon footprints, defined as the equivalent tons of CO_2 emissions produced by a particular set of activities. Food miles (Smith et al., 2005), the distance of food transport, is a critical factor determining the carbon footprint of food production. Edwards-Jones et al. (2008) criticized the popular assumption that "local is better," arguing that most analyses lack the empirical evidence needed for explicit life-cycle assessment. For example, they contend the distance considered within the range of "locality" is ambiguously interpreted, and criticize the widespread reliance on supply-chain-distance as the sole metric for evaluating food quality. They also question other ways we attempt to assess the nutritional quality and value of food. Their arguments highlight some weaknesses of the "local is better" assumption that we consider later. We stress that the important conceptual part of local plant consumption is the one that is usually least discussed—the use of native plants for novel agriculture.

The first step in commercializing any plant species is the search for relevant plants (**Figure 1**). The FAO estimates a mere 1% of available tree species have been studied for agricultural potential. As a matter of practical consideration, it is easier to search for agricultural potential under the bright light of traditional cultures. Ethnobotany, the study of native plant uses through the traditional knowledge of a local culture (Balick and Cox, 1996), had a significant contribution to the use of plants in the modern society, mainly for the pharmaceutical industry (Snader and McCloud, 1994). Ethnobotany uses socio-botanical surveys and questionnaires as a first step prior to phytochemical inspection. This practice is sometimes criticized for relying more on "primitive conception" through qualitative sociology inquiry than on "hard sciences" such as phytochemistry, pharmacology and agronomy. The search for new drugs is the main economic driver behind ethnobotanical studies, but increasing agrodiversity is as important as developing new drugs. Nevertheless, ethnobotanical studies have revealed important knowledge about native plants as food resource. Worth mentioning is a book by Daniel Moerman (1998) who listed the ten plants most commonly used for food by Native Americans: Common chokecherry (*Prunus virginiana*), Banana yucca (*Yucca baccata*), Saskatoon serviceberry (*Amelanchier alnifolia*), Honey mesquite (*Prosopis glandulosa*), Saguaro (*Carnegiea gigantea*), Broadleaf cattail (*Typha latifolia*), Corn (*Zea mays*), American red raspberry (*Rubus strigosus*), Salmonberry (*Rubus spectabilis*), and Thimbleberry (*Rubus parviflorus*). It is also worth mentioning that of all these plants, only the last four (corn and the three berries) are commercially used today in considerable scale. For additional examples of edible plants of the new world, and potential obstacles for commercialization, see **Table 1**.

Once a plant is identified as a novel food with good potential, its agricultural commercialization can be developed through two distinct strategies: one is establishing cultivated crops and the other is developing solutions for the efficient, cost-effective and ecologically sustainable gathering of native foods. Developing novel cultivated crops requires vast investments of time, knowledge, cash and patience for the long trial-and-error learning process that is required, which is why new crops are rare. Leaving the crop in its native habitat is a good solution, as illustrated globally with many plants. Coffee and cocoa—and to some extent tea, rice, coconut palm, avocado, date palm and pineapple—are examples of plants that are cultivated locally in their natural habitats and consumed globally. These systems challenge the concept of native plant use locally (see **Figure 2**): Is the global commercialization of a native cocoa plantation considered local food? Is it good for the local environment? We posit these extreme cases of native plant production, harvesting, transport, and consumption do not fit our thesis that promoting local food is neglected or necessarily beneficial. A related issue is use of native plants to improve existing conventional crops through back-to-nature crop breeding (Palmgren et al., 2015). This aspect is extensively studied and is not the focus of this review. Finally, natural systems are hard to mimic, and many species are impossible to domesticate. Yet, commercial use of wild plants can be economically plausible. Contemporary food gathering has great potential to expand the use of local plants, in concert with properly managing natural ecosystems, their resources and services, and improving plant gathering techniques at commercial scales.

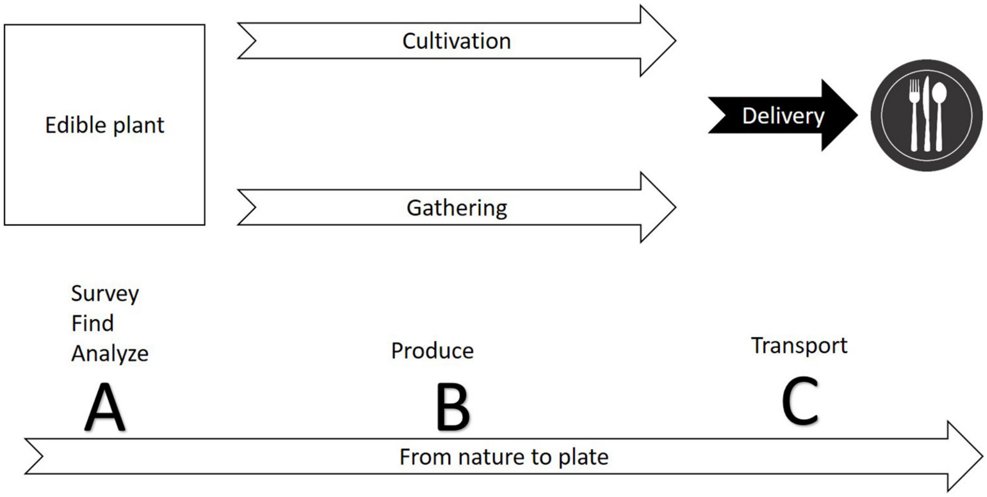

FIGURE 1 | A conceptual diagram illustrating the production cycle of edible plants from nature to plate. Commercialization of a new plant involves three phases: **(A)** finding a species with potential for safe use commercially—the species may be used already by indigenous people or may be totally novel; **(B)** establishing the technique for production through cultivation or gathering; **(C)** developing ways to harvest, store, and deliver the crop.

TABLE 1 | Examples of some edible plants of the new world, main consumption practice and possible reasons for commercialization hurdles.

Common name	Latin name	Origin	Consumed parts	Consumption	What challenges Commercialization	References
Açaí palm	*Euterpe oleracea*	Northern South America	Fruit and fruit pulp	Juice and juice as additive	Irregular supply, inconsistent quality, lack know-how in cultivation and processing	Pacheco-Palencia et al., 2007
Agave	*Agave tequilana*	Mexico, Southwestern United States	*Piña* (core)	Tequila, sweetener, potential biofuel	Traditionally grown in Mexico, local know-how and plant adaptations hinder wide distribution	Nobel, 1994; Davis et al., 2011
Banana yucca	*Yucca baccata*	South Western United States	Fruit	Fresh or heated		Moerman, 1998
Berries: Huckleberry, Raspberry, Salmonberry, Saskatoon serviceberry, Thimbleberry	*Vaccinium* spp., *Rubus strigosus*, *Rubus spectabilis*, *Amelanchier alnifolia, Rubus parviflorus*	North America	Fruits	Raw, dried, or as juice and jam	Yield fluctuations, cultivation barriers, fire management. Some cultivation developed in the last decade	Moerman, 1998; Barney, 2003
Biscuitroot	*Lomatium* Spp.	Western North America	Roots	Starchy food, cooked or grained to flour		Herzog, 2014
Bitterroot	*Lewisia rediviva*	Western North America	Roots	Traditional delicacy		Bandringa, 1999
California Black Oak (among other species)	*Quercus kelloggii*	Western United States	Acorns	Staple food for direct consumption, flour, oil	Long generation time, harvest is hard, expensive labor, process is needed (dry roasting, grinding, press)	Wolf, 1945; Ocean, 1993
Cassava	*Manihot esculenta*	West-central Brazil	USO	Starch for flour	Palate preferences limit it mainly to the southern hemisphere	Caballero-Arias, 2015
Chokecherry (bitter-berry)	*Prunus virginiana*	North America	Fruit	Cooked jelly, jam, syrup, and wine	Hard to collect or domesticate, need processing	Moerman, 1998
Common Sunflower	*Helianthus annuus*	North America	Seeds, flower bud	raw, roasted, cooked, dried, and ground, oil, coffee substitute	Commercially used	Heiser, 1976
Honey mesquite	*Prosopis glandulosa*	Southwestern United States and Northern Mexico	Pods	Starchy flour		Moerman, 1998
Indian rice grass	*Oryzopsis hymenoides*	North America	Seeds	Flour	Cultivated as Gluten-free grain	Dunmire and Tierney, 1997; Moreno et al., 2014
Joshua Tree	*Yucca brevifolia*	Arid southwestern United States	Seeds, flower buds	Oil	Product is not attractive to justify cultivation. Low indexes of cultural significance Stoffle et al., 1990	Wolf, 1945

(Continued)

TABLE 1 | Continued

Common name	Latin name	Origin	Consumed parts	Consumption	What challenges Commercialization	References
Mushrooms	*Tricholoma magnivelare, Marasmius oreades, Lycoperdon sp., Agaricus sp., Tremellodon sp., Latarius deliciosus, Lycoperdon perlatum, Morchella sp., Pleurotus sp., Ramaria sp.*		Sporocarp (fruit body)	Food, medicine	Lack of knowledge on gathering patterns, gathering systems are laborious	Richards, 1997
Pinweed	*Erodium cicutarium*	North America	Entire plant			Lovell, 1926
Pinyon Pine nuts	*Pinus monophylla, P. edulis, P. quadrifolia, P. remota, P. culminicola, P. johannis, P. orizabensis, P. cembroides*	New world	seeds	Raw food, pesto industry, oil,	Long generation time, harvest is hard, expensive labor, cheaper substitutes, seed extraction	Sharashkin and Gold, 2004
Saguaro	*Carnegiea gigantea*	Southwestern United States and Northern Mexico	Fruits	Fermented drink		Moerman, 1998
Sego lily	*Calochortus nuttallii*	Western United States	USO (underground storage organs), seeds, and flowers	starchy grain	Cultivated as ornamental plant, slow maturation of bulbs	Herzog, 2014
Wild onions and garlic	*Allium spp.*	Northern Hemisphere	Roots, leaves	Direct or cooked	Of hundreds species only handful are cultivated	Rabinowitch and Currah, 2002

An exhaustive list of all edible plants is out of the scope of this paper. Organized by alphabetically order of the common names.
We decided to focus in the New World, where agriculture history stretched back only several decades. Farmers in the Americas could chose to develop local plants, or utilize crops they imported from the Old World. This short history is emphasizing the tendency to prefer a limited number of local or imported crops rather than expanding agrodiversity by using local plants.

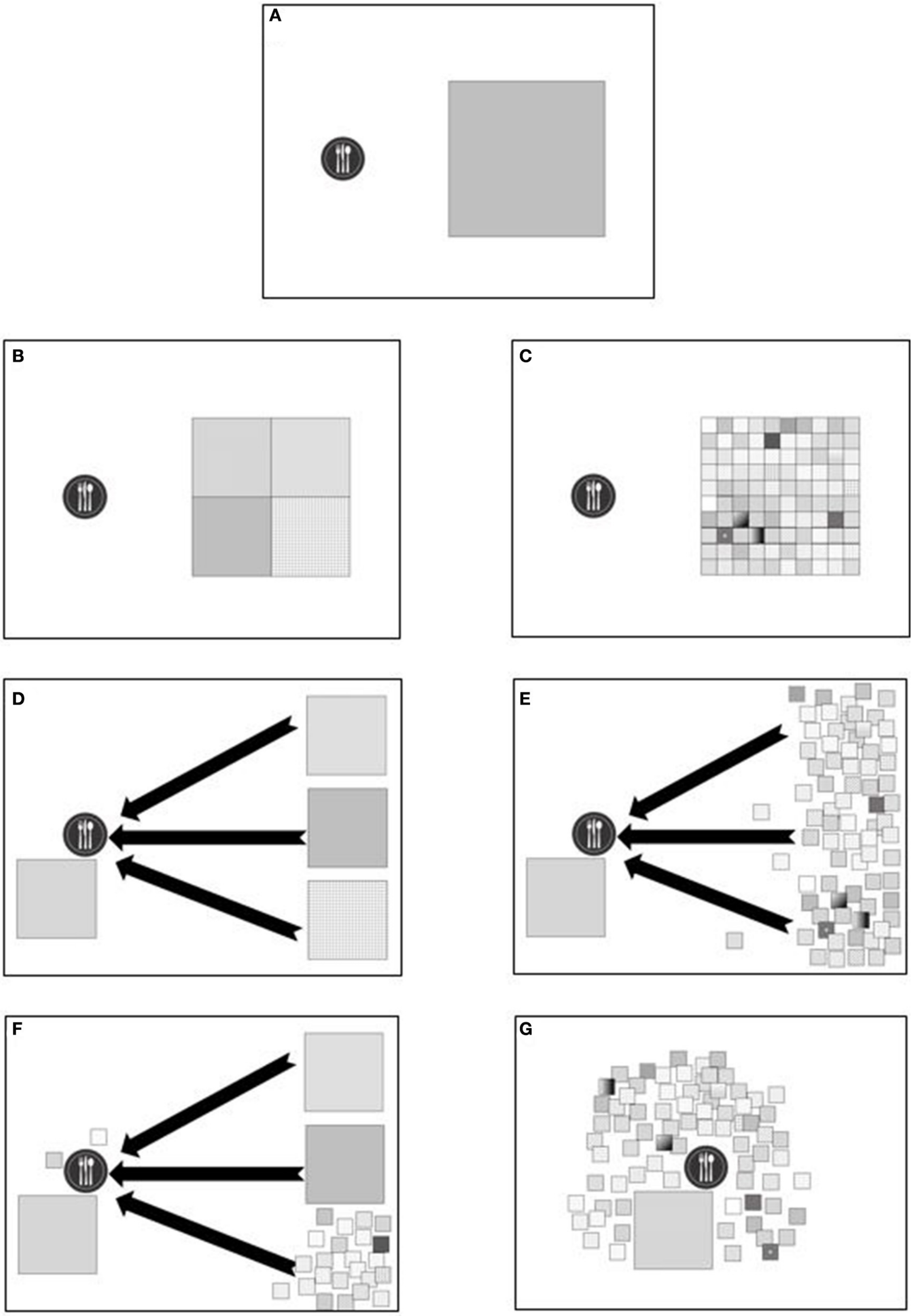

FIGURE 2 | Conceptual illustration of local food production, native plants and agro-diversity. Illustrations **(A–G)** describe the differences between native plant resources and local production, discussed further in the text. The plate represents a human community of consumers, and the squares represent their food resources. The area of each square represents its actual size and its relative contribution to the food supply of the consumers. A small square stands for a native plant that can

(Continued)

FIGURE 2 | supply food only when grown in its native range. The total area of squares is equal in all figures. **(A)** a community and its food demand; **(B)** a community reliant on four crops that each supply a quarter of the demand; **(C)** a community reliant on a high variety of plant resources, 25 times more diverse than community B; **(D)** a community fed by four plant resources, one of them is in proximity, the other three distant, demanding long chain of transports. Black arrows denote transport or food miles; **(E)** a community relying on one short-chain food resource and many small resources with long supply chains; **(F)** a mixture of one big resource in the vicinity of the community, two big and remote sources, and some small resources, most of them remote and two are local; **(G)** a community relying on a variety of locally grown plants.

THE BENEFITS AND ASSETS OF LOCAL FOOD PRODUCTION AND DEVELOPING NEW CROPS FROM NATIVE PLANTS

In the developing world, 10–15% of one billion hectares are farmed using traditional methods. Approximately 475 million people cultivate food in smallholder farms (FAO, 2016). Local production of food can reduce the carbon footprint of agriculture by lowering costs of production, shortening food miles, boosting local economies, and providing foods that are fresher and more nutritious for customers. The discipline of economic sociology links local food production to an increased sense of self-reliance or embeddedness of provisioning services, resulting in tighter social connectivity among individuals within communities and the landscapes they inhabit (Hinrichs, 2000). Indeed, in many industrial countries, the last decade saw proliferation of short-distance cooperative distribution and delivery programs such as community gardening and urban farming, farmers' markets, and various forms of community-supported agriculture including vegetable box delivery. These trends set the stage for native plants to develop into new biological resources that promote food diversity and crop resilience and enhance ecosystem services. The following is a more detailed list of the assets of local food production and utilization of native plants for food.

Advantages of practicing local food production:

1) Greater proximity of food production and consumption can lead to less waste and lower inputs of energy for transport, storage and preservatives, as well as support the recycling of plant nutrients, water and other inputs on site (for a review of the many inefficiencies in agriculture see Alexander et al., 2017). Local food supply helps to reduce food miles thus reducing carbon emissions (Cowell and Parkinson, 2003; Winter, 2003). For example, Coley et al. (2009) suggested that a round-trip distance of less than 6.7 km by each customer to purchase vegetables has a lower total carbon footprint than a system of regional storage and transport of the same food directly to the customer.
2) Locally grown crops supply fresher and potentially healthier food through reduced use of preservatives and reduced loss of nutritional value. Fresh food in short-chain production systems is less likely to be heavily processed. Processed food can negatively affect health by altering food preferences and appetitive states (Provenza et al., 2015).
3) Domestic production implies self-reliance with less imports (Little and Horowitz, 1987), which can promote societal sovereignty that may become essential if the availability and cost of fossil fuels make long-distance transport prohibitive. In addition, local production and delivery promote small-scale entrepreneurship, cultural diversity, sense of community, cultural and physiological relationships between people and seasonal availability of different foods.
4) At the ecosystem scale, the use of local plants can decrease the risk of exotic plant invasions that can adversely affect biodiversity (Cardinale et al., 2012). Compared with large-scale monoculture agriculture, local food production can reduce the spread of disease and the effects of invasive species. Transport infrastructure has an enormous impact on ecosystem fragmentation: the smaller the production-consumption circles, the smaller the impacts of fragmentation (Gehring and Swihart, 2003).

Advantages of using native plants and developing new crops:

1) Promoting genetic diversity. People have selected for growth over phytochemical richness in domesticated crops over the past 10,000 years (Provenza et al., 2015). In the process, domestication created a bottleneck of genetic diversity, as numerous genes were out selected (Vigouroux et al., 2005). Limited diversity of crops increases risks of disease and reduces potential for climate change adaptation. Native populations serve as a genetic bank that can enrich genetic diversity and phytochemical richness of crops, which in turn promotes resistance to adverse environmental conditions (Palmgren et al., 2015). The opportunity to develop and manage a greater array of native plants is critical to enhancing genetic diversity with potential for agricultural use.
2) Recruiting new local foods and crops is a way to diversify commercial uses, dietary options, and income for the local communities that rely on agriculture. Notably, the adaptation of local communities to climate changes will be critical for food security and poverty reduction (FAO, 2016).
3) Native plants are adapted to their homeland environment and thus better able to survive and produce high yields of phytochemically rich foods with fewer inputs including water, fertilization, and pest and disease control (Provenza et al., 2015).
4) Native plants are likely to mitigate soil erosion and conserve plant-microbe-soil interactions. Bacteria, fungi, endophytes and rhizobia in the rhizosphere are essential for health of plants and animals (Hawkes et al., 2007; Balestrini et al., 2015). These findings, which suggest we have underestimated the role of belowground interactions of plants with other organisms historically (Shelef et al., 2013), offer great potential to improve plant performance and crop yields (Drinkwater and Snapp, 2007). Mutualistic associations take time to

arise. Therefore, an optimal holobiome—sum total of all genomes in a living system—will be easier to maintain in the plant-rhizosphere-soil continuum developed in the location of origin than in a mixture of soil, plant and other inputs derived from different and distant locations not locally adapted. Plant diversity can also be maintained in the context of a shared holobiome, representing not only the genetic variety of the individual plant genomes but also the metagenome including associated fauna, such as the microorganisms in the rhizosphere and the phyllosphere, which contribute to efficient plant growth under evolving environmental conditions (Pérez-Jaramillo et al., 2016). Agricultural management based upon a metagenomics perspective can help to protect against emerging plant diseases and pests, and can potentially reduce the use of hazardous pesticides. In addition, decomposition processes are likely to occur faster and more efficiently with the home field advantage of native soil, plants, and herbivores (Ayres et al., 2009).

5) Incorporating native food plants as temporal and spatial intercrops for land management can help to maintain soil quality and prevent soil degradation. The no-tillage strategy depends on the availability of appropriate plants, often the local plants found in the field. Intercropping also helps to maintain soil quality and enhance nitrogen uptake (Eaglesham et al., 1981), repel herbivores and other enemies (Tonhasca and Byrne, 1994), reduce weeds (Liebman and Dyck, 1993) and offer a higher net income to farmers (Yildirim and Guvenc, 2005). Local plants as intercrops have two prominent advantages—local adaptation is likely to occur with little external inputs of water or fertilizers and the hazard of invasive species is avoided by using noninvasive species.
6) By augmenting local food production with native plants, people can enhance the diversity and resilience of existing crops, using genetic diversity of native progenitors or crop-recent relatives that preserve desired traits. In tomato, for example, wild species outperformed the elite varieties for total yield and soluble solids (15%), and fruit color and sugars (40%), as compared to the normal improvement of 1% achieved annually through traditional breeding (Bernacchi et al., 1998). Similar potential exists for the wild type gene banks of the main crops (Tanksley and McCouch, 1997). Cox et al. (2006) discuss the benefits of breeding and domesticating perennial crops, including enhanced diversity of perennial plants in native terrestrial biomes as opposed to monocultures of annual crops. They also emphasize that today no perennial crops produce adequate grain yields, though the perennial crops that have been developed tend to store more carbon and require less resources. Science can expedite processes that a few millennia ago took centuries to develop, including improving food quality and resilience, and breeding perennial crops has been initiated in wheat, sorghum, sunflower and wheatgrass (Cox et al., 2006). Diamond (2002) stresses that knowledge regarding the control of bitterness and astringency will allow selection for fruits that were not edible before, for example acorns.
7) Local agriculture and native plants can help reduce human conflicts, diminish exploitation of labor forces in developing countries and enhance fair trade. An interesting example is the cassava market. The starchy roots of cassava (*Manihot esculenta)*, native to Brazil, were expanded to a global production of nearly 270 m tones a year by 2014 (FAO). This drought-tolerant crop is popular in small stakeholder farms in rural areas of Latin America, Asia and Africa, (Henry and Gottret, 1996). It is a unique example of a native Brazilian plant that is successfully cultivated and globally distributed, yet used primarily for self-production in short-chain markets. On the other hand, quinoa illustrates the problems that can occur when a local species is sold on international markets. Jacobsen (2011) argued that increased demand for quinoa put too much stress on the environment in Bolivia, leading to diminished biodiversity and land health. Quinoa illustrates the complexity of defining "local food" in a global economy. This crop is grown in its natural homeland, due to biological constraints, similar to many other crops including coffee, tea, cocoa, spices and herbs. Once commercialized and distributed throughout international markets, the impact on the local farmers can be uplifting or devastating. Nevertheless, we argue that with fair trade awareness and market incentives the use of native plants can expand and diversify agricultural resources.
8) Native species can reduce negative impacts of introduced species. Invasive species often spread and damage the environment, threatening biodiversity, agriculture, and human health (Schmitz and Simberloff, 1997). Insect outbreaks transform ecosystems (Foucaud et al., 2010); mammalian population outbreaks damage ecosystems and risk human safety (Côté et al., 2004); and weeds adversely impact rangelands across the U.S. and worldwide at an alarming rate (DiTomaso, 2000; Duncan et al., 2004). Recently, the EU Council adopted regulations on preventing and managing invasive species (PE-CONS 70/14, 13266/14 ADD 1), suggesting that of 12,000 alien species in Europe, as many as 10–15% spread and cause damage, estimated at 12 billion Euro each year. Clearly, encouraging the production and use of local species could help to alleviate these issues.
9) Using native species can positively influence human health. The so-called Western diet has changed key nutritional characteristics of human diets worldwide, especially with the introduction of processed foods. In addition, the food industry has selected for fruits and vegetables of low palatability by favoring varieties that are less phytochemically rich than their wild ancestors (Robinson, 2013; Reeve et al., 2016). Agricultural practices further diminish phytochemical richness by increasing resource availability through fertigation with off-farm sources of nitrogen, phosphorus, and potassium. Primary and secondary compounds increase when plants are mildly stressed due to less availability of nutrients and water, but decrease when agricultural practices emphasize productivity and growth (Bryant et al., 1983; Coley et al., 1985). Expanding and diversifying use of native plants, in combination with cultural practices for preparing those foods, would add health-promoting phytochemicals to diets and nullify the apparent economic costs of such practices (Provenza et al., 2015). The use of native plants, some of which have been used by humans for centuries, will result in

vegetable foods that are highly nutritious, palatable and easily digested.

In summary, significant advantages accrue to using local plants to supplement food production, and through the phytochemical richness they possess, enhance human health (Provenza et al., 2015). In addition to enhancing diet diversity for people, enhanced use of local plants will diversify agricultural entrepreneurship and preserve genetic diversity so as to enhance crop endurance during stressful environmental conditions. Local species can reduce input investment and environmental conflicts. Even if local species are not economically relevant globally, maintaining a diversity of plants from different geographic regions is important locally. Diverse plant communities have myriad adaptations to environmental stressors, developed over thousands of years in response to adverse environmental conditions. Seed-bank collections can provide a genetic resource to grow plants in various environmental conditions in different geographic areas under changing climates (Dempewolf et al., 2014). Domestication of plants, one of the most influential processes in human history, resulted in vast socioeconomic improvements and human development. According to Harris and Hillman (1989), the main trends were increasing sedentism (settlement size and duration), population density, and social complexity from ranking to state formation. Domestication of new crops has nearly stopped, supplanted by plant varietal breeding (and genetic modification) of already domesticated species. This practice creates a genetic bottleneck. For example, the rich reservoir of wild tomato species has narrowed to a few genetically poor cultivated varieties of tomatoes (Bai and Lindhout, 2007). Miller and Tanksley (1990) estimated that less than 5% of wild tomatoes' genetic diversity is contained in the genomes of modern cultivars. The current presumption in research and practice is that agro-variability could be remunerated by introgression of adaptive traits from wild species to existing crops (Zamir, 2001) by researchers seeking to improve crop resistance to abiotic stress (Flowers, 2004; Tester and Bacic, 2005), disease (Johnson and Jellis, 2013), and herbivory (Chaudhary, 2013). With growing initiatives to improve agriculture through science and technology, expanding use of native plants as novel crops is calling for more attention. To do so, we must first learn the challenges of developing new crops. If the benefits of using local species outcompete the use of global crops, why are they not used more frequently? Here we present some of the main reasons.

OBSTACLES TO DOMESTICATING LOCAL PLANT SPECIES AND COMMERCIALIZING THEIR PRODUCTS

Despite the advantages, recruitment of new crops from native plants is extremely challenging. Several obstacles explain why relying on native plants to supplement our diets remains to be developed for the future, and is not yet a common practice:

1) Intensive agriculture selects for cash crops at the expense of developing new crops with lower environmental impacts. Existing crops are ready to use, whereas developing new crops is demanding and risky. Existing companies, families, machinery, roads and customers are all part of a well-known infrastructure for food production. Neither producers nor consumers are interested in leaving the familiar system to risk investing in new crops. Evolving from the familiar into the unfamiliar typically comes about only when people are under great duress (Massy, 2017).
2) Consumer acceptance of novel food is hard to predict. An interesting example is the acceptance of juice made of Açaí palm (*Euterpe oleracea*). The plant, native to Brazil and Trinidad, has a growing market as a healthy tropical juice commercially distributed in Europe and the USA. Sabbe et al. (2009) showed that consumer acceptance and purchase intention of the fruit juice was affected by interactions among many variables including socio-demographic characteristics, health-orientation, perception of health claim, and of course, to a large extent, taste experience. A rich body of literature is related to causes and consequences of "food neophobia," the fear of eating unfamiliar foods (see for example Dovey et al., 2008).
3) Domestication depends on financial investment and has high risk. This implies that modern domestication can flourish only with the strong support of policy makers and people with strong financial interests.
4) Regulatory barriers exist for developing new crops. New foods require the approval of government agencies. Proving that a new food is safe for all consumers is not an easy task. Only a handful of countries (e.g., Australia, Britain, USA, and France) possess the technical and procedural abilities to assess the risks of eating new foods. Most governments rely on protocols and lists of edible species produced in those countries. If the new food is not on those lists, regulators are unlikely to prioritize investments in the risky process of developing new crops, resulting in missed opportunities for the entrepreneurial development of new crops derived from native plant species.
5) In some countries, the use of local species may give rise to intellectual property concerns (Ahmed and Johnson, 2000), as indigenous communities may claim local plants and cultivation and gathering procedures as their sole property.
6) Exploiting indigenous peoples' rights (Lee, 2013) may hinder domestication efforts. Indigenous communities tend to protect their resources, which can cause conflicts when other people want to share their experience. Cultivating food that was formerly collected in the wild may require careful analysis of the effects of the new practice on rural farmers and harvesters (Stewart and Cole, 2005). The surging economy generated by the Açaí palm, for example, has negatively impacted local communities in the Eastern Amazon estuary. The intensification of Açaí forestry impacted land tenure systems, transportation systems, and social inequalities among the local Caboblu producers due to the growing demand from international urban centers (Brondízio et al., 2002).
7) Risk of overexploitation. Souther and McGraw (2014) predicted that climate warming (1°C, next 70 years) and harvest will result in high risk of extinction of American

ginseng (*Panax quinquefolius* L.). Similarly, local species are used in oil palm agriculture, but 60% of the oil palm plantation land use is at the expense of natural forests, threatening their unique biodiversity and many ecological services (Koh and Wilcove, 2008). Thus, the use of local species must involve a thorough study of the effects on ecosystems including species biology, carrying capacity and interactions with other species. Cultivating an over-harvested plant can provide strong conservation benefits while still providing food and income to indigenous populations, a strategy preferred by Tekinşen and Güner (2010), who study tubers of native Turkish orchids. The tubers of at least 30 species and 10 genera of the *Orchidaceae* family are traditionally collected to produce a local delicate hot drink known as "*Salep*," as well as, among other products, a savory stabilizer of ice cream. This high-quality local plant product has been traded in the Mediterranean region for centuries. Nevertheless, producing 1 kg of *Salep* requires thousands of dried tubers and irresponsible plant poaching exposed the orchid population to the risk of extinction—an estimated annual damage to 120 million wild *Salep* plants (Kreutz, 2002).

8) Biological barriers to domestication. Only a handful of plants have been successfully domesticated in the last centuries. They include strawberries, blueberries, macadamia, and pecan nuts, which all had negligible economical value as compared to ancient domesticated plants. An interesting example is the enormous effort invested attempting to domesticate truffles. The desert truffle *Terfezia boundieri* is associated with the host plant *Helianthemum sessiliflorum* (Turgeman et al., 2011). For decades, local Bedouin people have eaten the truffle, which has great potential as a gourmet food, highly valuable nutritionally and commercially (Kagan-Zur et al., 2013). Truffles could be a novel crop with low inputs (Kagan-Zur, 2001). Nevertheless, the complex symbiosis of this mycorrhizal system (Zaretsky et al., 2006) has not proved easy to domesticate and commercialize, despite several decades of research. The same is true with huckleberries (Barney, 2003). Another example, among many others, is the desert plant *Erodium crassifolium*, an edible tuber plant used traditionally by indigenous peoples (Batanouny, 2001), which was never commercialized despite the fact it could potentially serve as an energy source (carbohydrates) and a low input crop.

9) In addition to plant biology, some agro-technical issues must be addressed, even when a plant is successfully transferred from its native habitat to an agricultural field. The quality and quantities of plant products are affected by seasonality, climate, temperature, soil, nutrients and water supply. For example, secondary metabolites of plants are often the target of cultivation, as in the case of spices, tinctures and drinks. However, the production of secondary metabolites can be significantly altered when nutrient and water supply is insufficient (Gershenzon, 1984), or with seasonal changes (Grulova et al., 2015). Hence, finding the best conditions to develop a new cultivar demands ample amounts of trial and error, meaning vast investment of time, labor and resources. Commonly, harvesting fruits and other plant parts from naturally occurring stands and trees is more practical than cultivation and domestication (Barney, 2003). However, some masting species like acorns are subject to long reproductive maturity and episodic fruit production.

10) The use of local varieties may result in the disappearance of cultivars that support regenerative agriculture. For example, Oriental Wheat Triticum turanicum Jakubz (Grausgruber et al., 2005) is praised as a highly nutritious pure ancient stand. Avoiding the use of this cultivar just because it has expanded far from its area of origin (Anatolia, according to Gökgöl, 1961) would have contradicted many other aspects of promoting regenerative agriculture.

11) Once established, a new crop could rapidly spread and would not be a local crop anymore. The direct consequence is that a successful new crop could inhabit new places and become a well-established exotic and potentially invasive harmful species. This can be avoided if plants are used in their native range. For certain crops such as coffee, rice, and certain tropical fruits, biological barriers dictate that crops are used only in their home ranges.

UTILIZATION OF LOCAL PLANT SPECIES—THE CASE OF PINE NUT PRODUCTION IN THE WESTERN US

While export of agricultural products occurs globally, there are plenty of untapped local resources. For example, approximately 11 species of North American pinyon pine produce edible and highly nutritious nuts, with the most important being Colorado piñon (*Pinus edulis*), dominant throughout pinyon-juniper woodlands of the southwestern USA and Colorado Plateau, and singleleaf pinyon pine (*Pinus monophylla*), which is abundant throughout the Great Basin "cold desert" of Nevada and western Utah. Archeobotanical records have dated pine nut gathering in Utah to at least 7500 years before present (Rhode and Madsen, 1998). As climates warmed and some species moved north during the Holocene, the arrival of *P. monophylla* to the Great Basin approximately 6000 years ago provided a critical protein source that allowed people of the Middle Archaic period to extend their seasonal use patterns beyond the wetland habitats bordering pluvial lakes, into the surrounding uplands (Simms, 2008). Today, the same *Pinus* species cover large portions of western North America, estimated at approximately 56 million acres (Mitchell and Roberts, 1999), equivalent to 22.6 million hectares.

Although piñon pine nuts are more nutritious than many other tree nuts that are extensively cultivated in orchards—*P. edulis* is rich in oils and *P. monophylla* is rich in proteins and carbohydrates (Lanner, 1981)—pine nuts in the United States are harvested only locally and nut harvests are not commercially important. Yet large quantities of pine nuts are consumed each year in the United States, often serving as a key ingredient in pesto, salads and various Mediterranean dishes. Rich in unsaturated fatty acids, pine nuts are beneficial for controlling coronary heart disease through reduction of lipids in the circulatory system (Ryan et al., 2006). In a $100 million market over 80% of pine nuts consumed annually in the United States are imported mainly from eastern Asia (Russia and northeastern China; *Pinus koraiensis*) and Mediterranean Europe (*Pinus pinea*) (Sharashkin and Gold, 2004). As a result, massive collection of pine nuts in Russia and northeastern China continues to degrade the Korean pine broad-leaved forests (Ogureeva et al., 2012; Zhao et al., 2014), thousands of miles away from regions in North America and Europe where the nuts are consumed (Slaght, 2015).

Despite the advantages, developing a commercial, local pine nut industry in the western U.S. faces multiple challenges including:

1. Long generation time: reproductive maturity occurs at 25–50 years, with maximum seed production occurring at 75–100 years (Krugman and Jenkinson, 1974).
2. Episodic seed production: Good crop years of these masting species are highly variable in space and time, occurring every 4–7 years (Barger and Ffolliott, 1972). During drought periods, the frequency of good mast years can be reduced by as much as 40%, particularly when drought is associated with high late summer temperatures (Redmond et al., 2012).
3. Picking nuts is laborious work and access to nut-producing woodlands is often limited.
4. Potential competition with cultural users of pine nuts. Pine nut gathering remains important to native peoples in the region, and increased commercialization of the pine nut could come into conflict with such uses.
5. Potential ecological sustainability issues. Commercial pine nut harvesting could create competition for critical forage resources required by certain seed-caching wildlife species, including Pinyon Jay, Clark's Nutcracker, and several species of fossorial rodents (Vander Wall, 1997). Widespread seed harvesting could also negatively affect the regeneration potential of piñon pine populations, and hence resilience to episodic drought events that cause extensive overstory mortality (Redmond et al., in press).

Management of pinyon-juniper woodlands in the Western United States has not strongly considered the food value of pine nuts. In combination with recent drought events that have resulted in widespread tree mortality that threatens the long-term resilience of pinyon-juniper woodlands (Breshears et al., 2005; Redmond et al., in press), recent and planned management activities also threaten to reduce the availability of the pine nut resource. Pinyon-juniper woodlands are currently targeted for widespread tree removals across large areas of their distribution, particularly in the Great Basin. The objectives are to create forage for livestock and game mammals, to create or maintain habitat for sagebrush specialist species such as Greater Sage-Grouse, to provide woody fuels for bioenergy projects, to reduce fire risk, and to increase resilience to post-fire invasion of exotic annual grasses by fostering an understory of native perennial herbaceous species (Chambers et al., 2014). Ironically, extensive tree removal projects have occurred or are planned in many areas that were tree-dominated prior to Euro-American settlement, but were harvested in the late nineteenth Century to provide charcoal and woody fuels for mining-related activities (Young and Budy, 1979; Ko et al., 2011; Lanner and Frazier, 2011). Subsequent regrowth over the past 100–150 years is commonly viewed as an expansion of tree cover by human inhabitants of the region, whose generation time is much shorter than that of pinyon pines. In any case, many of the desired management objectives for fire risk reduction and conservation of understory plant species and the associated shrub-steppe habitats do not require complete woodland removal, and can be compatible with the goal of maintaining abundant pinyon pine seed production for wildlife and humans. Silvicultural methods, likely including uneven-aged management on favorable sites, can be further developed to promote drought-resilient, fire-resistant woodlands with a significant proportion of seed-producing trees (Gottfried and Severson, 1993; Page, 2008). Cone production in *Pinus pinea* can be increased by judicious thinning (Moreno-Fernandez et al., 2013).

One requires only a small stretch of the imagination to envision people in the Western United States meeting their demand for pine nuts through purchase from local harvesters, or by harvesting the nuts themselves when cones ripen in the autumn. This would greatly reduce the carbon footprint associated with pine nut importation, and would require no water use or fertilizer application, as piñon pines occur naturally under the driest conditions and in relatively nutrient-poor soils. Increased consumption of locally harvested pine nuts might also have the desirable effect of reducing the incidence of "pine nut syndrome" or "pine mouth". This condition is characterized by an annoying metallic taste that can linger in the mouth for multiple days, and that has been associated with consumption of *Pinus armandii*, an inedible pine species whose nuts are occasionally found mixed within pine nut batches that have been imported from Asia (Mikkelsen et al., 2014).

Despite all the good reasons, economic and environmental, to promote a local agriculture of pine nuts, we are still far from seeing considerable change from importing these nuts to developing local production. In a world motivated by short-term economic incentives, with nearly unlimited transportability of foods across the globe, most foods people eat are not produced locally. If costs for transport increase, due to rising costs of fossil fuels, that will drastically change the value of local food production and consumption.

FUTURE PROSPECTS OF LOCAL FOOD PRODUCTION

A recent call to rethink the research and development of food production urges us to nourish humanity more efficiently and improve the food disparity of a world in which 795 million people are undernourished and 2 billion adults are overweight or obese (Haddad et al., 2016). Haddad et al. (2016) discuss ten global research goals, two of which are closely related to our discussion. The first implies understanding the role of food-chain length. Ultimately, that would lead to an optimal mix of short-chain systems where high-quality food is produced and consumed nearby and long-chain systems where large quantities of food travel great distances (see **Figures 2F,G**). Second, they argue that to improve global food production we must analyze business incentives, mainly for private farmers, retailers and food processors. To help kick-start these activities, we contend that governments should offer more incentives for shorter food-chains by finding solutions to enhance diversity of uses of native plants. Awareness of consumers and farmers for the benefits of commercializing native species will play an important role. The local food movement, urban farming, production and consumption of pesticide-free healthy, nutritious, savory and sustainable food have attracted a great deal of attention in the last decade.

We refer here to agriculture as a more complex system than traditional cultivated crops. Agriculture has a strong impact on the environment: soil and water quality and quantity, deforestation, habitats and biodiversity, intensive farming, economic and social conditions in rural communities (Massy, 2017). The consequences can include the loss of biodiversity, accelerated land degradation, high fertilizer inputs, water contamination and the spread of pesticides hazardous to human health. Regenerative agriculture has arisen as a reaction to the negative effects of agriculture including impacts on land and resource management, humans and ecosystem interfaces. Agricultural practices can move from external-input farming to low-input practices (e.g., water, nutrients, pest control, land, energy) without significantly reducing production (Pittelkow et al., 2015). One of the greatest challenges for agriculture is to reduce the distances between crop production and food consumption. In some cases, this challenge can be met by using local species.

Recruiting native plants to develop cultivation of novel crops has great potential to establish new markets. This potential is countered by great challenges and enormous financial demands—lack of knowledge concerning unfamiliar species, the need for hybridization and agro technical improvements, sometimes with slow growing plants, and the risks associated with exchanging existing crops for uncertain income opportunities in an already conservative market. Some plant species are completely incompatible with any sort of domestication, or their cultivation requires an enormous investment of research, time and money. That is the case for slow growing species (e.g., many trees), plants with specific and narrow niche breadth (e.g., orchid tubers), and food sources that require complex biological interactions that are hard to mimic (i.e., edible mycorrhiza). Nevertheless, the success of some plants that are now harvested for commercial use (e.g., truffles, pine nuts, berries, spices, and herbs) demonstrate that modern food gathering is feasible. Food gathering may be improved in various ways, although many of them are not commonly practiced and deserve more attention. The first step is developing tools to find biological resources that are not used today, by expanding the strategy of ethnobotany, with its pros and cons. People also must continue to evolve ways to better manage naturally occurring plantations, a process that is site-specific. The last step is improving technological solutions for gathering, picking and processing wild fruits and other plant organs. Commercial gathering and developing new crops may balance each other, as the risk of overexploitation may be offset by mitigation of undesired plant invasions and overuse of agricultural inputs.

Local does not necessarily mean native, and using non-native foods grown, harvested, stored and delivered near the place of their consumption is advantageous. Native plants can complement these efforts. Native plants require lower inputs of water, nutrients, pest control and energy. Nevertheless, the long road to greater use of native species and local food production has many obstacles to overcome. Biological barriers to domestication are a challenge. In addition, global markets make it difficult to establish new crops. Other barriers include lack of financial incentives and investments, regulations, and agro-technical boundaries. Moreover, a successful new crop is likely to spread rapidly across the globe, losing its local value. Despite these challenges, the advantages of using native plants for food production are many. They include enabling diverse agriculture entrepreneurship, preserving interspecies crop and genetic diversity to enhance crop endurance in adverse environmental conditions, reducing inputs, reducing conflicts over indigenous land management, reducing environmental conflicts, and intercropping to improve land management.

CONCLUSION

To date, most research and practical efforts have been devoted to improving existing crops, rather than recruiting new, local species. We conclude that native food production should receive more attention in research and application to initiate and empower regenerative agriculture. Moving from monocultures to more diverse local crops, and domestication of new species, can conserve biological resources, and help to foster more sustainable agroecosystems. However, the use of native plants in local food production has not yet attained a high level of awareness. To reach an optimal balance between short- and long-chains of food production, shorter chains should be supported more vigorously and the evaluation of this balance should consider a more thorough-life-cycle analysis of food production (Edwards-Jones et al., 2008). A pivotal strategy to support more local sources of food production is to allocate more resources for improving harvesting of local plants.

AUTHOR CONTRIBUTIONS

All authors have made a substantial, direct and intellectual contribution to the work, and approved it for publication. OS initiated the work, PW elaborated on the case study of pine nuts, FP was a pivoting writer and improved articulation.

REFERENCES

Ahmed, A. K., and Johnson, K. A. (2000). Horticultural development of Australian native edible plants. *Aust. J. Bot.* 48, 417–426. doi: 10.1071/BT99042

Alexander, P., Brown, C., Arneth, A., Finnigan, J., Moran, D., and Rounsevell, M. D. (2017). Losses, inefficiencies and waste in the global food system. *Agric. Syst.* 153, 190–200. doi: 10.1016/j.agsy.2017.01.014

Ayres, E., Steltzer, H., Simmons, B. L., Simpson, R. T., Steinweg, J. M., Wallenstein, M. D., et al. (2009). Home-field advantage accelerates leaf litter decomposition in forests. *Soil Biol. Biochem.* 41, 606–610. doi: 10.1016/j.soilbio.2008.12.022

Bai, Y., and Lindhout, P. (2007). Domestication and breeding of tomatoes: what have we gained and what can we gain in the future?. *Ann. Bot.* 100, 1085–1094. doi: 10.1093/aob/mcm150

Balestrini, R., Lumini, E., Borriello, R., and Bianciotto, V. (2015). Plant-soil biota interactions. *Soil Microbiol. Ecol. Biochem.* 311–338. doi: 10.1016/B978-0-12-415955-6.00011-6

Balick, M. J., and Cox, P. A. (1996). *Plants, People, and Culture: The Science of Ethnobotany*. San Francisco, CA: W. H. Freeman & Company; Scientific American Library.

Bandringa, R. W. (1999). "The ethnobotany and descriptive ecology of bitterroot, Lewisia rediviva Pursh (Portulacaceae)", in *The Lower Thompson River Valley, British Columbia: a salient root food of the Nlaka'pamux First Nation*. Doctoral dissertation, University of British Columbia?

Barger, R. L., and Ffolliott, P. F. (1972). *Physical Characteristics and Utilization Potentials of Major Woodland Tree Species in Arizona*. Res. Pap. RM-83, Fort Collins, CO: U.S.Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station.

Barney, D. L. (2003). Prospects for domesticating western huckleberries. *Small Fruits Rev. 2*, 15–29. doi: 10.1300/J301v02n01_03

Batanouny, K. H. (ed.). (2001). "Adaptations that engender escape from drought," in *Plants in the Deserts of the Middle East* (Berlin; Heidelberg: Springer), 127–133.

Bernacchi, D., Beck-Bunn, T., Eshed, Y., Lopez, J., and Petiard, V., Uhlig, et al. (1998). Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersicon hirsutum*. *Theor. Appl. Genet.* 97, 381–397. doi: 10.1007/s001220050908

Boxall, A. B., Hardy, A., Beulke, S., Boucard, T., Burgin, L., Falloon, P. D., et al. (2009). Impacts of climate change on indirect human exposure to pathogens and chemicals from agriculture. *Environ. Health Perspect.* 117, 508–514. doi: 10.1289/ehp.0800084

Breshears, D. D., Cobb, N. S., Rich, P. M., Price, K. P., Allen, C. D., Balice, R. G., et al. (2005). Regional vegetation die-off in response to global-change-type drought. *Proc. Natl. Acad. Sci. U.S.A.* 102, 15144–15148. doi: 10.1073/pnas.0505734102

Brondízio, E. S., Safar, C. A., and Siqueira, A. D. (2002). The urban market of açaí fruit (*Euterpe oleracea* Mart.) and rural land use change: ethnographic insights into the role of price and land tenure constraining agricultural choices in the Amazon estuary. *Urban Ecosyst.* 6, 67–97. doi: 10.1023/A:1025966613562

Bryant, J. P., Chapin, I. I. I., F. S., and Klein, D. R. (1983). Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* 40, 357–368. doi: 10.2307/3544308

Caballero-Arias, H. (2015). "From bitter root to flat bread: Technology, food, and culinary transformations of cassava in the Venezuelan Amazon." in *Cooking Technology: Transformations in Culinary Practice in Mexico and Latin America*, ed S. I. Ayora-Diaz (London: Bloomsbury Academic) 41.

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., et al. (2012). Biodiversity loss and its impact on humanity. *Nature* 486, 59–67. doi: 10.1038/nature11148

Chambers, J. C., Miller, R. F., Board, D. I., Pyke, D. A., Roundy, B. A., Grace, J. B., et al. (2014). Resilience and resistance of sagebrush ecosystems: implications for state and transition models and management treatments. *Rangeland Ecol. Manage.* 67, 440–454. doi: 10.2111/REM-D-13-00074.1

Chaudhary, B. (2013). Plant domestication and resistance to herbivory. *Int. J. Plant Genomics* 2013:572784. doi: 10.1155/2013/572784

Coley, D., Howard, M., and Winter, M. (2009). Local food, food miles and carbon emissions: a comparison of farm shop and mass distribution approaches. *Food Policy* 34, 150–155. doi: 10.1016/j.foodpol.2008.11.001

Coley, P. D., Bryant, J. P., and Chapin, F. S. III. (1985). Resource availability and plant antiherbivore defense. *Science* 230, 895–900. doi: 10.1126/science.230.4728.895

Côté, S. D., Rooney, T. P., Tremblay, J.-P., Dussault, C., and Waller, D. M. (2004). Ecological impacts of deer overabundance. *Annu. Rev. Ecol. Evol. Syst.* 35, 113–147. doi: 10.1146/annurev.ecolsys.35.021103.105725

Cowell, S. J., and Parkinson, S. (2003). Localisation of UK food production: an analysis using land area and energy as indicators. *Agric. Ecosyst. Environ.* 94, 221–236. doi: 10.1016/S0167-8809(02)00024-5

Cox, T. S., Glover, J. D., Van Tassel, D. L., Cox, C. M., and DeHaan, L. R. (2006). Prospects for developing perennial grain crops. *Bioscience* 56, 649–659. doi: 10.1641/0006-3568(2006)56[649:PFDPGC2.0.CO;2]

Davis, S. C., Dohleman, F. G., and Long, S. P. (2011). The global potential for Agave as a biofuel feedstock. *GCB Bioenergy* 3, 68–78. doi: 10.1111/j.1757-1707.2010.01077.x

Dempewolf, H., Eastwood, R. J., Guarino, L., Khoury, C. K., Müller, J. V., and Toll, J. (2014). Adapting agriculture to climate change: a global initiative to collect, conserve, and use crop wild relatives. *Agroecol. Sust. Food.* 38, 369–377. doi: 10.1080/21683565.2013.870629

Diamond, J. (2002). Evolution, consequences and future of plant and animal domestication. *Nature* 418, 700–707. doi: 10.1038/nature01019

DiTomaso, J. M. (2000). Invasive weeds in rangelands: species, impacts, and management. *Weed Sci.* 48, 255–265. doi: 10.1614/0043-1745(2000)048[0255:IWIRSI]2.0.CO;2

Dovey, T. M., Staples, P. A., Gibson, E. L., and Halford, J. C. (2008). Food neophobia and 'picky/fussy'eating in children: a review. *Appetite* 50, 181–193. doi: 10.1016/j.appet.2007.09.009

Drewnowski, A., and Popkin, B. M. (1997). The nutrition transition: new trends in the global diet. *Nutr. Rev.* 55, 31–43. doi: 10.1111/j.1753-4887.1997.tb01593.x

Drinkwater, L., and Snapp, S. (2007). *Understanding and Managing the Rhizosphere in Agroecosystems*. Burlington, MA: Elsevier.

Duncan, C. A., Jachetta, J. J., Brown, M. L., Carrithers, V. F., Clark, J. K., Ditomaso, J. M., et al. (2004). Assessing the economic, environmental, and societal losses from invasive plants on rangeland and wildlands 1. *Weed Technol.* 18, 1411–1416. doi: 10.1614/0890-037X(2004)018[1411:ATEEAS]2.0.CO;2

Dunmire, W. W., and Tierney, G. D. (1997). *Wild Plants and Native Peoples of the Four Corners.* Santa Fe, NM: Museum of New Mexico Press 313.

Eaglesham, A., Ayanaba, A., Rao, V. R., and Eskew, D. (1981). Improving the nitrogen nutrition of maize by intercropping with cowpea. *Soil Biol. Biochem.* 13, 169–171. doi: 10.1016/0038-0717(81)90014-6

Edwards-Jones, G., Canals, L. M., Hounsome, N., Truninger, M., Koerber, G., Hounsome, B., et al. (2008). Testing the assertion that 'local food is best': the challenges of an evidence-based approach. *Trends Food Sci. Technol.* 19, 265–274. doi: 10.1016/j.tifs.2008.01.008

FAO (2016). *The State of Food and Agriculture 2016 (SOFA): Climate Change, Agriculture And Food Security*. Available online at: http://www.fao.org/publications/card/en/c/18679629-67bd-4030-818c-35b206d03f34/

Flowers, T. J. (2004). Improving crop salt tolerance. *J. Exp. Bot.* 55, 307–319. doi: 10.1093/jxb/erh003

Foucaud, J., Orivel, J., Loiseau, A., Delabie, J. H., Jourdan, H., Konghouleux, D., et al. (2010). Worldwide invasion by the little fire ant: routes of introduction and eco-evolutionary pathways. *Evol. Appl.* 3, 363–374. doi: 10.1111/j.1752-4571.2010.00119.x

Gehring, T. M., and Swihart, R. K. (2003). Body size, niche breadth, and ecologically scaled responses to habitat fragmentation: mammalian predators in an agricultural landscape. *Biol. Conserv.* 109, 283–295. doi: 10.1016/S0006-3207(02)00156-8

Gershenzon, J. (1984). "Changes in the levels of plant secondary metabolites under water and nutrient stres," in *Phytochemical Adaptations to Stress,* Vol. 18, eds B. N. Timmermann, C. Steelink, F. A. Loewus (Boston, MA: Springer), 273–320.

Gökgöl, M. (1961). Die iranischen Weizen. *Z. Pflanzenzüchtg* 45, 315–333.

Gottfried, G. J., and Severson, K. E. (1993). "Distribution and multiresource management of piñon-juniper woodlands in the southwestern United States," in *Managing Piñon-Juniper Ecosystems for Sustainability and Social Needs: Proceedings of the Symposium* (Santa Fe, New Mexico. Gen. Tech. Rep. RM-236. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station).

Grausgruber, H., Oberforster, M., Ghambashidze, G., and Ruckenbauer, P. (2005). Yield and agronomic traits of Khorasan wheat (*Triticum turanicum* Jakubz.). *Field Crops Res.* 91, 319–327. doi: 10.1016/j.fcr.2004.08.001

Grulova, D., De Martino, L., Mancini, E., Salamon, I., and De Feo, V. (2015). Seasonal variability of the main components in essential oil of Mentha× piperita L. *J. Sci. Food. Agric.* 95, 621–627. doi: 10.1002/jsfa.6802

Haddad, L., Hawkes, C., Webb, P., Thomas, S., Beddington, J., Waage, J., et al. (2016). A new global research agenda for food. *Nature* 540, 30–32. doi: 10.1038/540030a

Hammer, K., Arrowsmith, N., and Gladis, T. (2003). Agrobiodiversity with emphasis on plant genetic resources. *Naturwissenschaften* 90, 241–250. doi: 10.1007/s00114-003-0433-4

Harris, D. R., and Fuller, D. Q. (2014). *Agriculture: Definition and Overview, Encyclopedia of Global Archaeology.* New York, NY: Springer.

Harris, D. R., and Hillman, G. (1989). "An evolutionary continuum of people-plant interaction," in *Foraging and Farming: The Evolution of Plant Exploitation*, eds D. R. Harris and G. C. Hillman (London: Unwin Hyman Ltd.), 11–26.

Hawkes, C. V., DeAngelis, K. M., and Firestone, M. K. (2007). "Root interactions with soil microbial communities and processes," in *The Rhizosphere, an Ecological Perspective*, eds Z. G. Cardon and J. L. Whitbeck (New York, NY: Elsevier/Academic Press), 1–29.

Heiser, C. B. Jr. (1976). *The Sunflower*. Oklahoma, OK: University of Oklahoma Press.

Henry, G., and Gottret, M. V. (1996). *Global Cassava Trends: Reassessing the Crop's Future*. Cali, CO: Centro Internacional de Agricultura Tropical (CIAT), p. 45. Working document no. 157.

Herzog, N. M. (2014). Starch grain analysis in California and the Great Basin. *California Archaeol.* 6, 171–189. doi: 10.1179/1947461X14Z.00000000039

Hinrichs, C. C. (2000). Embeddedness and local food systems: notes on two types of direct agricultural market. *J. Rural Stud.* 16, 295–303. doi: 10.1016/S0743-0167(99)00063-7

Horrigan, L., Lawrence, R. S., and Walker, P. (2002). How sustainable agriculture can address the environmental and human health harms of industrial agriculture. *Environ. Health Perspect.* 110:445. doi: 10.1289/ehp.02110445

Howden, S. M., Soussana, J. F., Tubiello, F. N., Chhetri, N., Dunlop, M., and Meinke, H. (2007). Adapting agriculture to climate change. *Proc. Natl. Acad. Sci. U.S.A.* 104, 19691–19696. doi: 10.1073/pnas.0701890104

Jacobsen, S. E. (2011). The situation for quinoa and its production in southern Bolivia: from economic success to environmental disaster. *J. Agron. Crop Sci.* 197, 390–399. doi: 10.1111/j.1439-037X.2011.00475.x

Johnson, R., and Jellis, G. J. (eds.). (2013). *Breeding for Disease Resistance,* Vol. 1. New York, NY: Springer Science & Business Media.

Kagan-Zur, V. (2001). "Terfezias, a family of mycorrhizal edible mushrooms for arid zones," *Combating Desertification with Plants*, eds D. Pasternak, A. Schlissel (Boston, MA: Springer), 45.

Kagan-Zur, V., Roth-Bejerano, N., Sitrit, Y., and Morte, A. (eds.). (2013). *Desert Truffles: Phylogeny, Physiology, Distribution and Domestication,* Vol. 38. New York, NY: Springer Science & Business Media.

Ko, D. W., Sparrow, A. D., and Weisberg, P. J. (2011). Land-use legacy of historical tree harvesting for charcoal production in a semi-arid woodland. *For. Ecol. Manag.* 261, 1283–1292. doi: 10.1016/j.foreco.2011.01.007

Koh, L. P., and Wilcove, D. S. (2008). Is oil palm agriculture really destroying tropical biodiversity? *Conserv. Lett.* 1, 60–64. doi: 10.1111/j.1755-263X.2008.00011.x

Kreutz, K. A. J. (2002). "Türkiye'nin orkideleri. Salep, dondurma ve katliam," in *Yeşil Atlas*, eds G. Müdür et al. (Broken Arrow: DBR), 98–109.

Krugman, S. L., and Jenkinson, J. L., (1974). "Pinus L. Pine," in *Seeds of Woody Plants in the United States*, Agriculture Handbook 450, ed C. S. Schopmeyer (Washington, DC: U.S. Department of Agriculture), 598–638. Available online at: https://www.fs.fed.us/psw/publications/krugman/psw_1974_krugman002.pdf

Lanner, R. M. (1981). *The Piñon Pine: A Natural and Cultural History.* Reno, NV: University of Nevada Press.

Lanner, R. M., and Frazier, P. (2011). The historical stability of Nevada's pinyon-juniper forest. *Phytologia* 93, 360–387. Available online at: http://www.phytologia.org/uploads/2/3/4/2/23422706/933360-387lannerpinyonjunipernevada.pdf

Lee, L. S. (2013). Horticultural development of bush food plants and rights of Indigenous people as traditional custodians–the Australian Bush Tomato (*Solanum centrale*) example: a review. *Rangeland J.* 34, 359–373. doi: 10.1071/RJ12056

Lemaux, P. G. (2009). Genetically engineered plants and foods: a scientist's analysis of the issues (Part II). *Annu. Rev. Plant Biol.* 60, 511–559. doi: 10.1146/annurev.arplant.043008.092013

Liebman, M., and Dyck, E. (1993). Crop rotation and intercropping strategies for weed management. *Ecol. Appl.* 3, 92–122. doi: 10.2307/1941795

Little, P., and Horowitz, M. (1987). Subsistence crops are cash crops: some comments with reference to eastern Africa. *Hum. Organ.* 46, 254–258. doi: 10.17730/humo.46.3.58411820x8823725

Lovell, J. H. (1926). *Honey Plants of North America*. Medina, OH: A.I. Root Company.

Massy, C. (2017). *Call of the Reed Warbler: A New Agriculture–A New Earth*. Brisbane, QLD: University of Queensland Press.

Mikkelsen, A. A., Jessen, F., and Ballin, N. Z. (2014). Species determination of pine nuts in commercial samples causing pine nut syndrome. *Food Control* 40, 19–25. doi: 10.1016/j.foodcont.2013.11.030

Miller, J. C., and Tanksley, S. D. (1990). RFLP analysis of phylogenetic relationships and genetic variation in the genus Lycopersicon. *TAG Theor. Appl. Genet.* 80, 437–448. doi: 10.1007/BF00226743

Mitchell, J. E., and Roberts, Jr., T.C. (1999). "Distribution of pinyon-juniper in the western United States," *in Proceedings: Ecology and Management of Pinyon-Juniper Communities within the Interior West* (Ogden, UT: US Department of Agriculture, Forest Service, Rocky Mountain Research Station), 146–154.

Moerman, D. E. (1998). *Native American Ethnobotany*. Portland, OR: Timber Press.

Moreno, M. D. L., Comino, I., and Sousa, C. (2014). Alternative grains as potential raw material for gluten-free food development in the diet of celiac and gluten-sensitive patients. *Austin J. Nutri. Food Sci*. 2:1016.

Moreno-Fernandez, D., Canellas, I., Calama, R., Gordo, J., and Sanchez-Gonzalez, M. (2013). Thinning increases cone production of stone pine (*Pinus pinea* L.) stands in the Northern Plateau (Spain). *Ann. For. Sci.* 70, 761–768. doi: 10.1007/s13595-013-0319-3

Nobel, P. S. (1994). *Remarkable Agaves and Cacti*. Oxford, UK: Oxford University Press.

Ocean, S. (1993). *Acorns and eat'em. Ocean-Hose.* Oakland, CA: California Oak Foundation.

Ogureeva, G., Dudov, S., and Karimova, T. Y. (2012). Diversity and protection of Korean pine broad-leaved forests in the Manchurian Natural Area. *Contemp. Probl. Ecol.* 5, 621–632. doi: 10.1134/S1995425512070062

Pacheco-Palencia, L. A., Hawken, P., and Talcott, S. T. (2007). Phytochemical, antioxidant and pigment stability of açai (*Euterpe oleracea* Mart.) as affected by clarification, ascorbic acid fortification and storage. *Food Res. Int.* 40, 620–628. doi: 10.1016/j.foodres.2006.11.006

Page, D. H. (2008). "Preliminary thinning guidelines using stand density index for the maintenance of uneven-aged pinyon-juniper ecosystems," in *Ecology, Management, and Restoration of Piñon-Juniper and Ponderosa Pine Ecosystems: Combined Proceedings of the 2005*. St. George, Utah and 2006 Albuquerque, New Mexico workshops, Proceedings RMRS-P-51, US Department of Agriculture, Forest Service, Rocky Mountain Research Station; Fort Collins, CO, USA.

Palmgren, M. G., Edenbrandt, A. K., Vedel, S. E., Andersen, M. M., and Landes, X., Østerberg, J.T., et al. (2015). Are we ready for back-to-nature crop breeding? *Trends Plant Sci.* 20, 155–164. doi: 10.1016/j.tplants.2014.11.003

Pérez-Jaramillo, J. E., Mendes, R., and Raaijmakers, J. M. (2016). Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant Mol. Biol.* 90, 635–644. doi: 10.1007/s11103-015-0337-7

Pimentel, D., Zuniga, R., and Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol. Econo.* 52, 273–288. doi: 10.1016/j.ecolecon.2004.10.002

Pittelkow, C. M., Liang, X., Linquist, B. A., Van Groenigen, K. J., Lee, J., Lundy, M. E., et al. (2015). Productivity limits and potentials of the principles of conservation agriculture. *Nature* 517, 365–368. doi: 10.1038/nature13809

Provenza, F. D. (2008). What does it mean to be locally adapted and who cares anyway? *J. Anim. Sci.* 86, E271–E284. doi: 10.2527/jas.2007-0468

Provenza, F. D., Meuret, M., and Gregorini, P. (2015). Our landscapes, our livestock, ourselves: restoring broken linkages among plants, herbivores, and humans with diets that nourish and satiate. *Appetite* 95, 500–519. doi: 10.1016/j.appet.2015.08.004

Rabinowitch, H. D., and Currah, L. (eds.). (2002). *Allium Crop Science: Recent Advances.* Oxford, UK: CABI.

Redmond, M. D., Forcella, F., and Barger, N. N. (2012). Declines in pinyon pine cone production associated with regional warming. *Ecosphere* 3:120. doi: 10.1890/ES12-00306.1

Redmond, M. D., Weisberg, P. J., Cobb, N. S., and Clifford, M. J. (in press). Woodland resilience to regional drought: dominant controls on tree regeneration following overstorey mortality. *J. Ecol.*

Reeve, J. R., Hoagland, L. A., Villalba, J. J., Carr, P. M., Atucha, A., Cambardella, C., et al. (2016). Chapter Six-organic farming, soil health, and food quality: considering possible links. *Adv. Agron.* 137, 319–367. doi: 10.1016/bs.agron.2015.12.003

Rhode, D., and Madsen, D. B. (1998). Pine nut use in the early Holocene and beyond: the Danger Cave archaeobotanical record. *J. Archaeol. Sci.* 25, 1199–1210. doi: 10.1006/jasc.1998.0290

Richards, R. T. (1997). What the natives know. Wild mushrooms and forest health. *J. Forestry*. 95, 5–10.

Robinson, J. (2013). *Breeding the Nutrition Out of Our Food*. New York, NY: The New York Times.

Ryan, E., Galvin, K., O'connor, T., Maguire, A., and O'brien, N. (2006). Fatty acid profile, tocopherol, squalene and phytosterol content of brazil, pecan, pine, pistachio and cashew nuts. *Int. J. Food Sci. Nutr.* 57, 219–228. doi: 10.1080/09637480600768077

Sabbe, S., Verbeke, W., and Van Damme, P. (2009). Analysing the market environment for açaí (*Euterpe oleracea* Mart.) juices in Europe. *Fruits* 64, 273–284. doi: 10.1051/fruits/2009022

Schmitz, D. C., and Simberloff, D. (1997). Biological invasions: a growing threat. *Issues Sci. Technol.* 13, 33–40.

Sethi, S. (2015). *Bread, Wine, Chocolate: The Slow Loss of Foods We Love.* New York, NY: HarperCollins.

Shand, H. (2000). Biological meltdown: the loss of agricultural biodiversity. *Reimagine: Race Poverty Environ.* Available online at: http://www.reimaginerpe.org/node/921

Sharashkin, L., and Gold, M. (2004). "Pine nuts: species, products, markets, and potential for US production, Northern Nut Growers Association 95th Annual Report," in *Proceeding for the 95th Annual Meeting* (Columbia, MI).

Shelef, O., Fernández-Bayo, J., Sher, Y., Ancona, V., Slinn, H., and Achmon, Y. (2018). "Elucidating local food production to identify principles and challenges for sustainable agriculture," in *Sustainable Food Systems from Agriculture to Industry: Improving Production and Processing,* eds C. M. Galanakis (New York, NY: Elsevier-Academic Press), 416.

Shelef, O., Guy, O., Solowey, E., Kam, M., Degen, A. A., and Rachmilevitch, S. (2016). Domestication of plants for sustainable agriculture in drylands: experience from the Negev Desert. *Arid Land Res. Manag.* 30, 209–228. doi: 10.1080/15324982.2015.1089954

Shelef, O., Helman, Y., Behar, A., and Rachmilevitch, S. (2013). Tri-party underground symbiosis between a weevil, bacteria and a desert plant. *PLoS ONE* 8:e76588. doi: 10.1371/journal.pone.0076588

Simms, S. R. (2008). *Ancient Peoples of the Great Basin and Colorado Plateau.* Walnut Creek, CA: Left Coast Press, Inc.

Slaght, J. C. (2015). *Making Pesto? Hold the Pine Nuts.* New York, NY: The New York Times.

Smith, A., Watkiss, P., Tweddle, G., McKinnon, A., Browne, M., Hunt, A., et al. (2005). *The Validity of Food Miles as an Indicator of Sustainable Development.* Final Report. Report ED50254. Aea Technology Environment.

Snader, M., and McCloud, T. G. (1994). Ethnobotany and drug discovery: the experience of the US National Cancer Institute. *Ethnobot. Search New Drugs* 185:178.

Souther, S., and McGraw, J. B. (2014). Synergistic effects of climate change and harvest on extinction risk of American ginseng. *Ecol. Appl.* 24, 1463–1477. doi: 10.1890/13-0653.1

Stewart, K. M., and Cole, D. (2005). The commercial harvest of devil's claw (Harpagophytum spp.) in southern Africa: the devil's in the details. *J. Ethnopharmacol.* 100, 225–236. doi: 10.1016/j.jep.2005.07.004

Stoffle, R. W., Halmo, D. B., Evans, M., and Olmsted, J. E. (1990). Calculating the cultural significance of American Indian plants: Paiute and Shoshone ethnobotany at Yucca Mountain, Nevada. *Am. Anthropol.* 92, 416–432. doi: 10.1525/aa.1990.92.2.02a00100

Tanksley, S. D., and McCouch, S. R. (1997). Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* 277, 1063–1066. doi: 10.1126/science.277.5329.1063

Tekinşen, K. K., and Güner, A. (2010). Chemical composition and physicochemical properties of tubera salep produced from some *Orchidaceae* species. *Food Chem.* 121, 468–471. doi: 10.1016/j.foodchem.2009.12.066

Tester, M., and Bacic, A. (2005). Abiotic stress tolerance in grasses. From model plants to crop plants. *Plant Physiol.* 137, 791–793. doi: 10.1104/pp.104.900138

Tonhasca, A., and Byrne, D. N. (1994). The effects of crop diversification on herbivorous insects: a meta-analysis approach. *Ecol. Entomol.* 19, 239–244. doi: 10.1111/j.1365-2311.1994.tb00415.x

Turgeman, T., Asher, J. B., Roth-Bejerano, N., Kagan-Zur, V., Kapulnik, Y., and Sitrit, Y. (2011). Mycorrhizal association between the desert truffle *Terfezia boudieri* and *Helianthemum sessiliflorum* alters plant physiology and fitness to arid conditions. *Mycorrhiza* 21, 623–630. doi: 10.1007/s00572-011-0369-z

Vander Wall, S. B. (1997). Dispersal of singleleaf piñon pine (*Pinus monophylla*) by seed-caching rodents. *J. Mammal*. 78, 181–191. doi: 10.2307/1382651

Vigouroux, Y., Mitchell, S., Matsuoka, Y., Hamblin, M., Kresovich, S., Smith, J. S. C., et al. (2005). An analysis of genetic diversity across the maize genome using microsatellites. *Genetics* 169, 1617–1630. doi: 10.1534/genetics.104.032086

Winter, M. (2003). Embeddedness, the new food economy and defensive localism. *J. Rural Stud.* 19, 23–32. doi: 10.1016/S0743-0167(02)00053-0

Wolf, C. B. (1945). *California Wild Tree Crops*. California, CA: Rancho Santa Ana Botanic Garden. Record Number: 19456601349.

Yildirim, E., and Guvenc, I. (2005). Intercropping based on cauliflower: more productive, profitable and highly sustainable. *Eur. J. Agron.* 22, 11–18. doi: 10.1016/j.eja.2003.11.003

Young, J. A., and Budy, J. D. (1979). Historical use of Nevada's pinyon-juniper woodlands. *J. For. History* 23, 112–121. doi: 10.2307/4004663

Zamir, D. (2001). OPINION: Improving plant breeding with exotic genetic libraries. *Nat. Rev. Genet.* 2:983. doi: 10.1038/35103590

Zaretsky, M., Sitrit, Y., Mills, D., Roth-Bejerano, N., and Kagan-Zur, V. (2006). Differential expression of fungal genes at preinfection and mycorrhiza establishment between *Terfezia boudieri* isolates and *Cistus incanus* hairy root clones. *New Phytol.* 171, 837–846. doi: 10.1111/j.1469-8137.2006.01791.x

Zhao, F., He, H., Dai, L., and Yang, J. (2014). Effects of human disturbances on Korean pine coverage and age structure at a landscape scale in Northeast China. *Ecol. Eng.* 71, 375–379. doi: 10.1016/j.ecoleng.2014.07.072

Zohary, D., Hopf, M., and Weiss, E. (2012). *Domestication of Plants in the Old World: The Origin and Spread of Domesticated Plants in Southwest Asia, Europe, and The Mediterranean Basin*. Oxford: Oxford University Press on Demand.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Plant Science

REVIEW
published: 25 October 2017
doi: 10.3389/fpls.2017.01845

Capparis spinosa L. in A Systematic Review: A Xerophilous Species of Multi Values and Promising Potentialities for Agrosystems under the Threat of Global Warming

Stephanie Chedraoui[1,2], Alain Abi-Rizk[2], Marc El-Beyrouthy[2], Lamis Chalak[3], Naim Ouaini[2] and Loïc Rajjou[1]**

[1] IJPB, Institut Jean-Pierre Bourgin (INRA, AgroParisTech, CNRS, Université Paris-Saclay), Saclay Plant Sciences (SPS)-RD10, Versailles, France, [2] Faculty of Agricultural and Food Science, Holy Spirit University of Kaslik, Jounieh, Lebanon, [3] Faculty of Agricultural Sciences, Lebanese University, Beirut, Lebanon

Caper (*Capparis spinosa* L.) is a xerophytic shrub with a remarkable adaptability to harsh environments. This plant species is of great interest for its medicinal/pharmacological properties and its culinary uses. Its phytochemical importance relies on many bioactive components present in different organs and its cultivation can be of considerable economic value. Moreover, taxonomic identification of *C. spinosa* L. has been difficult due to its wide heterogeneity, and many authors fell into confusion due to the scarcity of genetic studies. The present review summarizes information concerning *C. spinosa* L. including agronomic performance, botanical description, taxonomical approaches, traditional pharmacological uses, phytochemical evaluation and genetic studies. This knowledge represents an important tool for further research studies and agronomic development on this indigenous species with respect to the emerging climatic change in the Eastern Mediterranean countries. Indeed, this world region is particularly under the threat of global warming and it appears necessary to rethink agricultural systems to adapt them to current and futures challenging environmental conditions. *Capparis spinosa* L. could be a part of this approach. So, this review presents a state of the art considering caper as a potential interesting crop under arid or semi-arid regions (such as Eastern Mediterranean countries) within the climate change context. The aim is to raise awareness in the scientific community (geneticists, physiologists, ecophysiologists, agronomists, …) about the caper strengths and interest to the development of this shrub as a crop.

Keywords: *Capparis spinosa* L., drought tolerance, cultivation, agronomy, taxonomy, genetic analysis, phytochemical, traditional use

Edited by:
José Manuel Mirás-Avalos,
Centro de Edafología y Biología
Aplicada del Segura (CSIC), Spain

Reviewed by:
Pinarosa Avato,
Università degli Studi di Bari Aldo Moro, Italy
Lyudmila Petrova Simova-Stoilova,
Institute of Plant Physiology and Genetics (BAS), Bulgaria

***Correspondence:**
Stephanie Chedraoui
stephaniechedraoui@hotmail.com
Loïc Rajjou
loic.rajjou@agroparistech.fr

Specialty section:
This article was submitted to Agroecology and Land Use Systems, a section of the journal Frontiers in Plant Science

Received: *13 June 2017*
Accepted: *10 October 2017*
Published: *25 October 2017*

Citation:
Chedraoui S, Abi-Rizk A, El-Beyrouthy M, Chalak L, Ouaini N and Rajjou L (2017) Capparis spinosa L. in A Systematic Review: A Xerophilous Species of Multi Values and Promising Potentialities for Agrosystems under the Threat of Global Warming. Front. Plant Sci. 8:1845. doi: 10.3389/fpls.2017.01845

INTRODUCTION

In a world likely to be challenged by the threat of global warming, it is expected to observe negative effects on growth and reproductive success of plants. The Mediterranean region has been pointed out as a climate change hot spot by the IPCC (Intergovernmental Panel on Climate Change; http://www.ipcc.ch; Pachauri et al., 2014). Evidences of substantial impact on agricultural production are already occurring. High temperatures, heat waves and drought stress leading to loss in plant productivity might result in an inability to ensure global food security (Bita and Gerats, 2013; Ray et al., 2015). For instance, wheat crop yields fell by 25–35% with a 3–4°C rise in temperature in the Middle East (Ortiz et al., 2008). Various molecular, cellular, physiological and morphological damages have been observed under elevated temperatures, leading to a decrease in plant growth (Vollenweider and Günthardt-Goerg, 2005; Hatfield and Prueger, 2015; Ohama et al., 2017). In many cases, aridity, excessive heat and elevated CO_2 cause modifications in respiration and photosynthesis, leading to a reduced plant life cycle and a loss in plant productivity (Prasad et al., 2008; Yamori et al., 2014; Xu et al., 2015).

Nevertheless, the introduction of stress tolerant crops and cultivars in agrosystems is not a rapid process due to the long delays between laboratory research and validation of field trials. Such crops might constitute an efficient way to cope with the foreseeable nutritional needs and to promote a sustainable agriculture (Thiry et al., 2016). In this context, this review gives attention to a xerophilous crop, well adapted to drought and of promising potentialities namely caper (*Capparis spinosa* L.). Caper is a Mediterranean shrub known for its edible flower buds and fruits pickled in salt and vinegar. This species possesses strong characteristics of adaptation to the regions displaying fluctuating climate and is a candidate for being domesticated to maintain and promote agriculture in regions subject to extreme climate change and affected by hyper-aridity. The advantages of using such xerophilous species include their moderate water requirements, a high potential for genetic improvement, local knowledge and know-how on this plant material and an existing global trade chain for the use of plant products. Perennial plantations of caper could contribute to preserve water in the soil for a longer period of time and can help to maintain sustainable agroecosystems. Such shrubs protect the soil from sunlight, limiting high soil temperatures and thus regulating the microclimate. By comparison with other desert plants, caper has a high water use efficiency (WUE) and a remarkable ability to search and absorb water from its environment (particularly in soil depths) thanks to an extensive root system and a very high root/stem ratio (Zuo et al., 2012; Gan et al., 2013). This root system is very effective for water retention during scattered rainfall events, providing suitable conditions for soil fauna and microbiota development. Caper plantations can be associated with annual plants (e.g., vegetables, grassland plants, medicinal herbs) to improve biodiversity and provide multiple benefits (Solowey, 2010). In addition, it has a considerable economic importance through the uses of its roots, buds and fruits in many food and pharmaceutical industries (Sher and Alyemeni, 2010). *C. spinosa* L. has an aesthetic blossom and a sweet-scented flower, thus it is used as an ornamental plant for gardens and walls as well for terraces exposed to sun. It requires no watering and can be grown in poor soils or even stones (Gan et al., 2013). At the agronomic level, this species has led to great financial returns from its cultivation due to its resistance to environmental stresses and its enormous ethnobotanical and pharmaceutical importance, as well as its content in bioactive agents having high nutritional value and great efficacy in the manufacture of medicines and cosmetics. Nevertheless, in the East Mediterranean countries, *C. spinosa* has not yet been sufficiently exploited due to the scarcity of buds consumption at the local level (Chalak et al., 2007).

Few studies have reviewed *C. spinosa* focusing on the plant nutritional quality, food and medicinal uses, phytochemistry, ethnopharmacology, biological activities and cultivation (Rivera et al., 2003; Sozzi and Vicente, 2006; Tlili et al., 2011a; Gull et al., 2015; Nabavi et al., 2016). *C. spinosa* displays huge agro-based potentialities and a highly demand for exploitation due to a diversified international market. Today, it seems necessary to focus on the possibility of selection and improvement of this specie and to develop more intensive research to promote this crop, especially in the east Mediterranean countries. Actually, the impacts of climate change are already being felt by the Arab region (UNEP/ROWA, 2015). Rural communities of this region are the first to be vulnerable to such changes. This could be overcome by exploiting and enlarging the cultivation of existing well adapted flora and by the development of crops highly tolerant to drought and heat stress. The awareness in agro-biodiversity for selecting the development of *C. spinosa* as a multipurpose crop that proved to have better resistance to drought and harsh environmental conditions is a significant need to alleviate climate change effects in agro-ecosystems of East Mediterranean region.

ORIGIN AND DISTRIBUTION

Origin and Discovery

The *Capparis spinosa* Linnaeus (1753: 503) group belongs to the *Capparis* genus *sect. Capparis* created and described by Carolus Linnaeus in his book "species Plantarum" (Inocencio et al., 2006). The genus *Capparis* belongs to the Capparidaceae family, closely related to Brassicaceae (Hall et al., 2002; Inocencio et al., 2006) and includes 350 species of tropical or subtropical origin, many of them distributed in the Mediterranean regions (Fici, 2001; Inocencio et al., 2006). *C. spinosa* was described as a hybrid between *C. orientalis and C. sicula* (Rivera et al., 2002). Caper is the English common name of this genus and it is also known by different names, e.g., Kabbar (Arab), câprier (French), and Alcaparro (Spain) (Zohary, 1960; Heywood, 1964; Jacobs, 1965; Inocencio et al., 2006; Saadaoui et al., 2007). Archaeological discoveries from an Old-World Paleolithic site in Egypt suggested *Capparis* spp. consumption since 17,000 years ago (Hillman, 1989; Hansen, 1991). Seed of *C. spinosa* L. were found at Tell es-Sawwan (Iraq, 5800 BC) and in the Yanghai Tombs of Turpan District in Xingjiang-China (2800 B.C.) (Renfrew, 1973; Jiang et al., 2007). The plant was used since ancient Greeks, Hebrews and Romans at Tell es Sweyhat-Syria. Pickled Capers consumption dates back to the Bronze Age. (Van and Bakker-Heeres, 1988; Sozzi, 2001). In the Middle East, Zohary regarded *Capparis* as a native flora distributed in Africa and south-western Asia (Zohary, 1960), whereas Jacobs suggested that the Malaysian and Australian *C. spinosa* were introduced by humans (Jacobs, 1960).

Geographic Distribution

Capparis spinosa grows naturally from the Atlantic coast of the Canary Island and Morocco to the Black Sea, in Crimea and Armenia, and to the east side of the Caspian Sea and Iran (Alkire, 1998; Inocencio et al., 2002). It is spread in North Africa, Europe, West Asia, Afghanistan, and Australia (Willis, 1988). This plant might have aroused in the tropics, and then extended to the Mediterranean basin and Central Asia (Zohary, 1960). Different subspecies and varieties have specific geographic distributions. *C. spinosa* subsp. *spinosa* is distributed in Southern Europe, northern Africa including Sahara, Arabic peninsula, and Middle East to China. *C. spinosa* subsp. *rupestris* is widespread in France, Italy, Spain, Slovenia, Malta, Croatia and Albania and also reported in Turkey, Greece, Algeria, Libya and Tunisia (Inocencio et al., 2006; Fici, 2015; see **Figure 1**). The Mediterranean regions might be harshly affected by global warming, leading to extensive effects on agroecosystems and crop production. A particular attention should be paid to plants adapted to arid conditions for being used in agricultural systems under the current climate change scenario.

BOTANICAL AND TAXONOMIC STUDIES

Botanical Description

Species belonging to the genus *Capparis* have plesiomorphic features (Fici, 2001). Some available literature treated the botanical description of *Capparis spinosa* and reported the polymorphic aspects of this species and the high degree of heterogeneity in its morphological characters (Post, 1932; Zohary, 1960; Mouterde, 1968; Higton and Akeroyd, 1991; Legua et al., 2013). The latter being slightly zygomorphic, abaxial sepal not galeate or slightly galeate with numerous stamens (Inocencio et al., 2006).

The species *C. spinosa* is a winter-deciduous perennial shrub. It is erect, precumbent or pendulous with branches being unramified or multi-ramified, green, red or yellow, attending 4 m long. Twigs are tortuous or straight, with or without simple hairs. Stipules are somewhat curved, straight, setaceous or spreading, antrorse or retrorse, orange, yellow or green, reaching 6 mm long. Leaf stipules may be formed into spines, granting it the name "spinosa." Leaves are rounded, or ovate, lanceloate or oblong, ellipticial or obordate with an obtuse, tapering, acute or cordate base and an acute, rounded, obcordate, truncate or obtuse apex. Leaf veins are prominent or not. Leaf texture can be glabrous, pubescent and very dense. Petiole is grooved or entire, 0–2 cm. Flowers are solitary, somewhat zygomorphic mainly noctoflorous. Four white or white-pinkish petals, oblong, obovate or rounded-ovate. Stamens are numerous with filaments up to 5 cm. Gynophore is 3–6 mm long. Fruit is ellipsoidal, obovate or oblong. Seeds are numerous and reddish-brown (Inocencio et al., 2006; Fici, 2014). Additionally, physiological capacities enabling adaptation of *C. spinosa* to drought conditions were ascertained. The plant might change its leaf, stem and root structure when facing dry areas. The xylem and fibro-vascular systems increase and the transit region between the root and stem enlarges in order to boost water absorption and storage capacity (Gan et al., 2013).

Taxonomic Description

Taxonomic studies based on the shrub leaf and flower phenotypes revealed a complex variation pattern within variants of *C. spinosa* on different landmasses (Zohary, 1960). Consequently, this made the identification of the *C. spinosa* group very complicated in the Mediterranean region. Many taxa at various ranks of classification have been described in the Middle East (Zohary, 1960; Maire, 1965; Inocencio et al., 2006; Danin, 2010).

A previous study indicated that *C. spinosa* is morphologically related to *C. sicula* Duhamel as well to *C. orientalis* Duhamel and overlaps with the latter (Inocencio et al., 2005). Recently a taxonomic revision has been conducted by Fici (2014, 2015) on the *C. spinosa* group widespread from the Mediterranean to central Asia. *C. spinosa* is recognized as a single species and is represented by four subspecies (i.e., *C. spinosa* subsp. *spinosa;* *C. spinosa* subsp. *rupestris*; *C. spinosa* subsp. *cordifolia*; *C. spinosa* subsp. *himalayensis). C. spinosa* subsp. *spinosa* is widely distributed eastwards from the Mediterranean to China and Nepal, showing inherited traits and great level of heterogeneity. Within this subspecies, some varieties are identified, namely var. *herbacea* and var. *atlantica*. *C. spinosa* subsp. *rupestris* is less diversified and more similar to the tropical lineage. Two varieties were also recognized, var. *ovate* and var. *myrtifolia*.

A more recent study investigated *C. spinosa* forms distributed in the Paleotropis, Australia and in a few tropical areas of northern-eastern Africa and southern Asia. Two original nomenclatures are proposed, i.e., C. *spinosa* subsp. *cordifolia* comb. et stat. nov. and *C. spinosa* subsp. *himalayensis* stat. nov. (Fici, 2015).

GENETIC DIVERSITY

Capparis spinosa shows considerable morphological variation due to various factors such as phenotypic plasticity, eco-geographical differentiation, topographical modifications, and hybridization processes promoting the presence of intermediate phenotypes. This high variability suggests chaotic complex structure within wild forms of *C. spinosa*. The pure morphological approaches based solely on qualitative and quantitative vegetative characters have led to much confusion in the taxonomy of *C. spinosa,* with misidentification of the taxon and erroneous classification of the different varieties. Therefore, research that deals with molecular data has greatly complemented morphological classifications and has helped in revealing the phylogenetic relationships, with different eco/biotypes and the evolutionary trends of this species. At present, a few number of studies reported molecular data in studying the taxonomy of *C. spinosa* and its genetic profile (**Table 1**).

FIGURE 1 | Distribution of *Capparis spinosa* L. (●) over the Mediterranean Basin (Adapted from Inocencio et al., 2006).

TABLE 1 | Genetic data available for the *Capparis spinosa* L. group in the Mediterranean and Near East.

References	Geographic origin	Markers	Results
Wang et al., 2016	China	cpDNA	Genetic differentiation by vicariance.
Liu et al., 2015	China	ISSR	Genetic clustering linked to geographic factors.
Al-Safadi et al., 2014	Syria	IRAP & ISSR	Genetic distinction between *Capparis* sp. with possible hybrid origin.
Silvestre et al., 2014	Sicily-Itlay	ISSR	Genetic discrimination between two subspecies.
Ozbek and Kara, 2013	Turkey	RAPD	Genetic differentiation of five varieties.
Bhoyar et al., 2012	India	RAPD and ISSR	Geographical distribution and genetic variation are correlated.
Nosrati et al., 2012	Azerbaijan Iran	RAPD	Genetic variation in small population is lower than that in large population.
Saifi et al., 2011	Morocco	ISSR	Genetic distance partially related to geographical distances.
Moubasher et al., 2011	Egypt	ISSR	Three varieties of *C. spinosa*. var. inermis suggested as independent species.
Inocencio et al., 2005	Spain, Morocco, Syria	AFLP	*C. spinosa* is a hybrid resulting from *C. orientalis* with introgression from *C. sicula*.
Khouildi et al., 2000	Tunisia, Central Italy	RAPD	Genetic variation is related to environmental factors rather than geographical distances.

Based on Amplified Fragment Length Polymorphism (AFLP) a low genetic distance was revealed among *Capparis* sp. (i.e., *C. spinosa*, *C. orientalis*, *C. sicula, C. aegyptia,* and *C. ovata*) from Spain, Morocco and Syria (Inocencio et al., 2005). About 50% of polymorphic frequency was revealed between *C. orientalis*, *C. spinosa* and *C. sicula* and a low consistency of *C. spinosa,* with 2% unique bands was marked. A possible hybrid origin of *C. spinosa* was suggested, comprising cultivars from different lineages of *C. orientalis* with some introgression from *C. sicula* thus a greater genetic influence from *C. orientalis* due to the unfrequented presence of *C. sicula* in the studied area (Balearic Islands) (Inocencio et al., 2005).

In Egypt, the taxonomic identity among and within species of the genus *Capparis* using Random Amplified Polymorphism DNA (RAPD) was conducted by Moubasher et al. (2011). Eight polymorphic RAPD markers were generated. A considerable genetic variation was identified and revealed the presence of three varieties of *C. spinosa*: var. *spinosa*, var. *canescens*, var. *deserti* and one inermis type. *C. spinosa* var. *inermis* was closer genetically to *C. sinaica* than to *C. spinosa* var. *spinosa, C. spinosa* var. *canescens,* and *C. spinosa* var. *deserti*. Thus *C. spinosa* var. *inermis* was suggested to be treated as independent species.

The genetic assessment of Moroccan capers by Inter Simple Sequence Repeat (ISSR) revealed 98.89% distinct profiles based on the geographic origin and indicated remarkable phenotypic plasticity linked to the ecological area and environment (Saifi et al., 2011). This might be explained by a low level of gene flow due to the fragmentation of habitats of these populations that leads to accumulate significant genetic differences (Inocencio et al., 2005). The genetic study of Azerbi and Iranian Capers using RAPD markers indicated no correlation between genetic variation and geographical distances among populations (Nosrati et al., 2012). Nevertheless, the same study revealed that those genetic distances were significantly lower in small populations than those in large populations with a percentage of polymorphic RAPDs bands ranging from 42 to 67% in small-sized populations and from 70 to 81% in large-sized populations. Moreover, 32.83% of total genetic variation was shared among populations while 67.17% restricted to within-populations, indicating an important fragmentation of habitats in this region.

Bhoyar et al. (2012) analyzed the genetic variability of *C. spinosa* populations growing in the trans-Himalayan region in India for adaptation to high altitude, by using both RAPDs and ISSRs markers. ISSRs were more efficient for detecting polymorphism in caper where microsatellites containing the repeated di-nucleotides (AG)n, (AC)n, (TG)n, (GA)n, and trinucleotides (ACC)n, and (GGC)n were frequent in caper. Geographical distribution and genetic variation were correlated, which can be explained as a sign of a longstanding pattern of restricted gene flow (Bhoyar et al., 2012).

In Turkey, Ozbek and Kara (2013) differentiated five varieties: *C. spinosa* var. *spinosa*, var. a*egyptia* and var. *canescens*, and *Capparis ovate* Desf. var. *palaestina*, and var. *herbacea*. Ten RAPD primers produced 98 loci, 73 of which were polymorphic with 87.42% total genetic variation. Hypothesis of the effect of population size on genetic diversity was confirmed as well as the relation between eco-geographical factors and genetic diversity affecting the number of effective alleles.

Silvestre et al. (2014) investigated capers growing in Sicily and the surrounding islets of Lampedusa, Pantelleria and Salina using ISSR markers. The results strongly supported morphological analysis and discriminated between the two subspecies *spinosa* and *rupestris,* indicating that genetic diversity can be related to environmental conditions rather than geographical distances. On the other hand, intermediate phenotypes showed hybridization between the two taxa for almost 80% in contact zones while cultivated biotypes presented genetic affinity to subsp. *rupestris*.

A recent study conducted in Syria correlated the morphological traits to the genetic differentiation and to the geographical distribution of *Capparis* species, using Inter Retro-transposon Amplified Polymorphism (IRAP), ISSR and combined data of IRAP+ISSR. The percentages of polymorphism recorded were 71, 82, and 75%, respectively for the three techniques. A clear separation was revealed among *C. spinosa* L., *C. aegyptia* Lam, and *C. sicula* Duh. Nevertheless, two samples could not be identified and were found at an intermediate position between *C. sicula* and *C. spinosa* indicating a possible hybrid origin between these two species (Al-Safadi et al., 2014).

The first genetic analysis of Chinese *Capparis spinosa* populations revealed the classification of the three distinct groups geographically separated and showed high genetic diversity using ISSR markers (Liu et al., 2015). In Western Himalayas, Tianshan Mountains and adjacent desert regions, vicariance phenomenon was suggested to explain genetic clades of *C. spinosa* identified based on three chloroplast DNA (cpDNA) fragments (Wang et al., 2016).

CULTIVATION AND PRODUCTION

Environmental Conditions

Capparis spinosa L. is a species of arid and semi-arid climate zones and is well known as a highly drought tolerant plant. It is one of a few species that grow and flower in summer in arid regions. In the Mediterranean basin, it is free of competition for water with other species (Rhizopoulou et al., 1997; Rhizopoulou and Psaras, 2003). It requires a semi-arid climate with mean annual temperatures over 14°C and mean annual rainfall not less than 200 mm. It is adapted to xeric areas, therefore, it can bear up water stress without any manifestation, and resists strong winds and temperatures exceeding 40°C in dry Mediterranean summers (Sozzi and Vicente, 2006). Moreover, caper survives winters in the form of stump; yet, frost can be disturbing during its vegetative period. It is usually grown at low altitudes even though some plants were found even over 1,000 m above sea-level (Barbera, 1991; Chalak et al., 2007).

C. spinosa was described as both a rupicolous and a stenohydric plant (Rhizopoulou et al., 1997). Stenohydric plants have not developed dehydration avoidance to as a degree as in desiccation-tolerant organisms such as resurrection plants. Caper plant adapts to calcareous soils or moderate percentages of clay (González, 1973). It has an efficient root system associated with nitrogen fixing bacteria that allows the growth in soils with poor fertility (Andrade et al., 1997). It also tolerates salty, sandy, or rocky soils, with low amount of organic matter as in India (Ahmed, 1986; Kala and Mathur, 2002). It prefers saline and halophytic habitats (Al-Yemeni and Zayed, 1999). Caper is also wildly grown in wall joints and in antique monuments (Barbera, 1991; Chalak et al., 2007).

C. spinosa has low flammability thus might be used for cutting down wild forest fires which are Mediterranean climate characteristics (Neyisci, 1987). *C. spinosa* is utilized for landscaping, it reduces erosions along steep rocky slopes, highways, sands dunes or fragile semiarid ecosystems (Faran, 2014). *C. spinosa* is a promising species due to its potential use in agroforestry and its ability to protect land in Mediterranean countries (Sher et al., 2012).

Ecophysiological Aspects and Adaptation Traits

The xeromorphic features of *C. spinosa* have been highlighted in several studies (Rhizopoulou, 1990; Rhizopoulou and Psaras, 2003; Sakcali et al., 2008; Wang et al., 2016).

Anatomical adaptations to aridity include root, stem, leaf and flower features. As mentioned above, a major aspect that may explain the high resistance of wild *C. spinosa* to drought concerns its extremely deep root system (Özkahraman, 1997). Caper root system represents 62.5% of the total plant biomass after 4–5 months of growth (Sozzi, 2001; Gan et al., 2013). Roots also excrete acidic compounds that can perforate rocks and cracks to reach water resources (Oppenheimer, 1960). In addition, the xylem vessels in stems are extremely well developed in *C. spinosa,* leading to an efficient hydraulic conductivity (Psaras and Sofroniou, 1999; Levizou et al., 2004). It is worth noting that the thick cortical layers in tap and fibrous roots and a swollen transfer region are able to store water and protect fibro-vascular bundle against damage under drought conditions (Gan et al., 2013).

At the leaf level, thick, small and multi-layered mesophyll cells were also found in *C. spinosa*. The small leaf intercellular air space percentage of 15% and the thick terminal epidermal cell walls are characteristic traits of xerophytes. Moreover, the wax-like and water-repellent cutin covering the epidermis and the shapely trichomes help the growing of *C. spinosa* in arid areas (Li et al., 2007). The well-developed sclerenchymatic tissue and the differentiated palisade parenchyma allow to maintain the protection of *C. spinosa* leaves against irreversible damages during severe water stress (Stefanou and Manetas, 1997; Rotondi et al., 2003). Stomata are the main channels for transpiration and are widely and evenly distributed across both leaf surfaces and are able to stay opened a full day. The opening of the stomata promotes evapotranspiration and has a strong cooling effect on leaf temperature in desert environments. Stomata were also found on the adaxial and abaxial surfaces of the petals and vacuolated parenchyma cells with large intercellular space. The membrane fluidity is influenced by the presence of unsaturated fatty acids, identified as major components of lipids in petals (Rhizopoulou et al., 2006). Under stress conditions, unsaturated fatty acids contribute to maintain membrane fluidity and its physiological functions. These traits offer a competitive advantage to this species.

The growth period and blooming of *C. spinosa* can occur entirely during dry and hot summers in the Mediterranean. It has been reported that the blooming of this shrub is not affected by severe water deficit (Vardar and Ahmed, 1972; Sheikh, 1976; Rhizopoulou and Psaras, 2003). Furthermore, high solar irradiance is very efficiently used by *C. spinosa* without any symptoms of photoinhibition. This photosynthetic performance makes *C. spinosa* a suitable candidate for being grown in drought areas, while most plants have minimum growth rates (Levizou et al., 2004).

Seed Propagation

One gram of fruit contains between 150 and 160 seeds (Gorini, 1981). Seeds are obtained by fruit rubbing followed by washing and drying in the shade (Sozzi and Vicente, 2006). Seed germination is the method of propagation mostly adopted for caper plant. The germination performance of caper seeds is poor due to a high dormancy and a low longevity. Seed viability is about 2 years when kept at 4°C and low relative humidity. Sprouted seeds are obtained after 25–50 days (Barbera, 1991). This traditional technique strongly limited by a low germination rate has been used in Argentina (Sozzi and Chiesa, 1995), Armenia (Ziroyan, 1980), Cyprus (Orphanos, 1983), India (Singh et al., 1992), Italy (Barbera and Di Lorenzo, 1984), Spain (Lorente and Vicente, 1985; Pascual et al., 2003), and USA (Bond, 1990).

The poor caper seed propagation is due to the weak germination capacity and to the hard coat of the seeds; therefore, the tough structure of the seed and the mucilage developing when placed in contact with water could limit the diffusion of oxygen to the embryo (Barbera, 1991; Bahrani et al., 2008). Indeed, the seed vigor (including speed and rate of germination) is affected by the maturity of the seeds, the fruit position and weight (Pascual et al., 2003). Different treatments are requested to overcome the prevailing dormancy to improve the germination (Sozzi and Chiesa, 1995). Among them, mechanical scarification (sand paper, ultrasound etc.), cold stratification, soaking in concentrated sulfuric acid (H_2SO_4), 0.2% KNO_3, gibberellins (GA_{4+7} and GA_3) and manipulation of the environmental conditions (light/dark, temperature) were efficient to promote caper seed germination.

Asexual Propagation

Use of stem cutting for propagation pays the serious rooting problems but has the advantages of avoiding high variability in terms of production and stability of quality traits. Vegetative propagation of caper allows to obtain numerous individuals from a limited number of plants. Stem cuttings can be obtained from hardwood, semi-hardwood or softwood (herbaceous) segments (Güleryüz et al., 2009). Hardwood cuttings vary in length from 1 to 50 cm and from 1 to 2.5 cm in diameter. Stems can be collected on February and March, treated with fungicides (e.g., captan or captafol) and then stratified outdoors or at 3–4°C and finally covered with sand or plastic (Lorente and Vicente, 1985). Semi-hardwood cuttings can be collected and planted on August and September, but low survival rates (under 30%) have been observed (Barbera, 1991). Softwood cuttings increase rooting percentage; they are collected and prepared on April (germination period) with basal or subterminal cuttings more successful than the terminal ones. Stem cuttings are planted under a mist system with heat that is believed to have a positive effect on rooting as well as dipping the cutting basal into auxin solution (1,500–3,000 mg/L) (Pilone, 1990). Hardwood cuttings do not seem to be influenced by hormonal treatments, whereas softwood cuttings gave 83% rooting percentages when treated with α-naphtaleneacetic acid (NNA) (Lorente and Vicente, 1985).

Propagation by grafting is a less adopted method for caper; however, it was carried out in Spain with acceptable results using bark grafting in planting (60% rooting) (Barbera, 1991) and could offer very interesting perspectives to develop caper hybrids (Zhou and Liu, 2015). *In vitro* propagation was successful from nodal shoot segments. Rodriguez et al. (1990) showed that 6-benzylaminopurine enhanced clusters proliferation when combined with indoleacetic acid and GA_3. Gamma irradiation stimulated growth of shoots up to 200% and increased shoot rooting percentage from 75 to 100% according to Al-Safadi and Elias (2011). The *in vitro* micropropagation of *C. spinosa* was reported in several countries (Salem et al., 2001; Chalak et al., 2003; Caglar et al., 2005; Musallam et al., 2010; Carra et al., 2011, 2012). Chalak and Elbitar (2006) described a protocol for the micropropagation of a Lebanese morphotype (*C. spinosa* subsp. *rupestris*) using single nodal cuttings. High rates of shootlets rooting response (92%) was obtained after 4 h pulse treatment period in darkness with auxin, followed by culture on solid half strength Murashige and Skoog basal medium. Development of a tissue culture system is a promising approach to identify high-yielding lines. Micropropagation protocols for caper could be useful and efficient in producing desirable seedlings for transplanting.

Cultivation, Practices, and Productivity

Caper plant phenology was reported using the BBCH scale (Biologische Bundesanstalt, Bundessortenamt, and CHemical industry) describing nine principal growth stages (Legua et al., 2013). The main traits of interest for cultivated caper bush are: high productivity, long stems, short internodes and high node fertility, dark green spherical flower buds with close non-pubescent bracts and late opening, oval fruit with light green pericarp and few seeds, absence of stipular spines, easy stalk separation to simplify harvest and postharvest operations, capacity for asexual reproduction and resistance to biotic and abiotic stresses (Barbera, 1991).

Caper is a spontaneously growing plant, though it is cultivated in several Mediterranean countries. It has already developed traits to survive new climate conditions. Therefore, its cultivation can help in adapting agricultural management to climate constraints in most Mediterranean regions (Howden et al., 2007).

C. spinosa is known as an economic plant in Australia and tends to spread in Latin America. The economic importance of caper has led to an increase in yield and production level. Specialized cultivation of caper started around 1970 in Spain and Italy, with a maximum of about 4,000 and 1,000 ha in cultivation, respectively in the 1990s. World caper production is estimated around 15–20,000 tons/year and the global trade concerns about 60 countries. Actually, Morocco and Turkey are the leading world producers and exporters (Infantino et al., 2007). Cultivation of caper is recorded in Spain, Italy and France, especially the Mediterranean island of Pantelleria, the Aeolian island of Salina and Sicily, where several local cultivars and ethnovarieties are known (Inocencio et al., 2006).

The most important Spanish cultivars (biotypes) are "Común" or "del País" and "Mallorquina" (Lorente and Vicente, 1985). Italian commercial biotypes are "Nocellara" (a cultivar within *C. orientalis*), and "Nocella." Other Italian biotypes are "Ciavulara," "Testa di lucertola," "Spinoso of Pantelleria" and "Spinoso of Salina" (a cultivar within *C. sicula* subsp. *sicula)* (Barbera, 1991). "Redona," "Roses," "De las Muradas," "FiguesSeques," and "Peluda" are cultivated in a lower amount in the Balearic Islands: (Rivera et al., 1999). Nevertheless, caper cultivation is mostly restricted to *C. spinosa* but also the commercial product known as "Capers" is actually being obtained from the cultivated *C. spinosa*, *C. orientalis* and *C. sicula*, in addition to intermediate biotypes having an identical genetic constitution (Inocencio et al., 2005).

Caper bush is cultivated mostly in non-irrigated lands. Despite its ability to grow in drought conditions, irrigation is especially important during the first year when the caper bush is highly sensitive to water stress (Sozzi and Vicente, 2006). Moldboard plowing and harrowing are usual practices prior to caper cultivation (Lorente and Vicente, 1985).

Nursery plants, propagated as seedlings or rooted cuttings, are maintained in nursery row during the dormant season. Transplanting, either bare-root or containerized, takes place after the last frosts and is carried out by hand (Sozzi and Vicente, 2006).

Square/rectangle and hedgerow planting designs are used. Spacing is determined according to the fertility of the soil, the resistance of the biotype, the equipment to be used and the irrigation method employed. Bush spacing of 2.5 × 2.5 m, or 2.5 × 2 m, 3 × 3 m, 4 × 4 or 5 × 5 m are satisfactory (Barbera and Di Lorenzo, 1984; Bounous and Barone, 1989). Caper bush cultivation can also be associated with vine (as in Pantelleria, Italy), olives (as in Salina, Italy) or almonds (as in south Spain) (Barbera, 1991).

Harvest is the heaviest operation of Caper production. It may represent 2/3 of the total labor as it is done manually. Harvest is difficult and time-consuming due to the dropping branches, the presence of stipular spines in some biotypes, the small diameter of flower buds and the high temperatures and solar radiation during summer under Mediterranean climate. Yields of flower buds increase with age, from 1 to 9 kg/plant/year. A maximum yield is expected in the 4th year; however, caper bush yields are highly variable depending on the age, growing environment, cultural practices and biotype.

Pests and Diseases

Capparis spinosa is not very sensitive to pests and pathogens when growing in wilderness (Sozzi and Vicente, 2006). Caper diseases have never been considered as limiting factors for this crop, probably because of the low production density. However, Caper can be attacked by wide range of species including insects, viruses and fungi (Infantino et al., 2007; **Table 2**).

Economic Value

The main economic importance of caper lays in dealing with flower buds, generally known in the market under the name of "capers" or "caper berry" which are the subject of considerable trade at an international level. Global caper production progressively increased at an annual growth rate of 6%. About 60 countries trade capers and the USA is considered as the most important consumer where the price reaches 25 US$/kg (ready for consumption). In the Balkans region, total production costs of caper represent less than 10% only of its selling price in the US markets. In Tunisia, the species is associated to a high socio-economic value especially for the rural farmers in the Northern country. The Chinese are earning an annual profit of 3 million US$ from this single specie (Saadaoui et al., 2011). More recently, *C. spinosa* is suggested to uplift the socio-economic level in the Kingdom of Saudi Arabia, in Lebanon, Syria and other Mediterranean countries (Sher and Alyemeni, 2010).

PHYTOCHEMICAL COMPOSITION AND ACTIVITIES

Extracts

Capparis spinosa has been investigated for its biochemical contents, which are affected by multiple factors such as geographical and environmental conditions, harvest date and size, preservation procedures, genotype, and processing methods of extraction (Sozzi and Vicente, 2006; Tlili et al., 2010). Capers are rich in phenolic compounds and flavonoids as reported in several studies (**Table 3**). Such secondary metabolites generally play a role in abiotic stress responses widely associated with tolerance to heat (Wahid, 2007). For instance, total phenolics ranged from 21.42 to 27.62 mg Gallic Acid Equivalent (GAE)/g of dry weight (DW) in caper leaves methanol extract taken from different sites in India. Caper leaves aqueous extract from Tunisia recorded total phenolics of 33.55 mg GAE/g DW and buds aqueous extracts contained 67.29 mg GAE/g DW, while

TABLE 2 | Vulnerability of _Capparis spinosa_ L. to pests and diseases.

Category	**Pathogen**	**Plant parts affected**	**Damages**	**Control**	**References**
Virus	Caper Latent Virus (CapLV) Eggplant Mottled Dwarf Virus (EMDV)	Leaves	• Asymptomatic • Clearing, yellowing veins • Necrosis • Curling leaves • Shortened internodes • Severe dwarfing • Decreasing yields.	• Understanding the epidemiology of each caper viruses • Developing certification protocol for virus testing • Obtaining small-scale production for virus-free seedlings	Ciferri, 1949; Di Franco and Gallitelli, 1985; Gallitelli and Di Franco, 1987; Adams et al., 2004; Tomassoli et al., 2005; Infantino et al., 2007
	Cucumber Mosaic Virus (CMV)		• Mosaic leaves • Chlorosis • Mottled leaves • Vein banding • Yellow Spots		
	Co-infection CMV and EMDV or CapLV		• Thickening, malformation of leaves • Stunting of the plant		
Fungi	_Fusarium_ spp.		• Rotting of cuttings • Damping-off of seedlings	• Avoidance of excessive watering • Use of steril soil	Lorente and Vicente, 1985
	Sclerotium rolfsii	Branches	• Yellowing and wilting of branches • Death of affected shoots	• Removal of crop debris • Weed control • Reduction of stress factors	Infantino et al., 2006
	Leveillula taurica	Leaves, petioles, branches	• Chlorosis • Necrosis • Defoliation • Production of conidiophores	• Sulphur-based fungicides • Humidity reduction	Gupta and Bhardwaj, 1998; Kavak, 2004; Infantino et al., 2007
	Albugo capparidis	Mainly leaves and flowers	• Whitre rust • Hypertrophy of leaves, flowers, peduncles • Floral abortion.	• Destroying infected plants	Ciferri, 1949; Infantino et al., 2007
Insect Pests	*_Acalles barbarus_ Lucas	Roots	• Slender mines in the woods	–	Liotta, 1977
	*_Phyllotreta latevittata Kutsch_	Leaves	Circular gouges	–	Longo, 1996
	**_Bagrada hilaris_ (Burmeister) Nezara viridula (L.) Eurydema ventrale Kol. Eurydema ornata L. Holcostethus punctatus L. Carpocoris lunula F.	Leaves, buds, fruits	• Yellowing spots and chlorosis • Hollowing out plant parts • Deformation	• Insecticides (pyrethroids, organophosphates, cabamates) • Some cultural practices like breaking uo the groundand destroying residues of alternative host plants	Colazza et al., 2004
	***: _Bemisia tabaci_ _Aleurolobus niloticus_ _Priesner and Hosny Brevicoryne brassicae (L.)_ _Aspidiotus nerii Bouchè_ _Planococcus citri Risso_	Leaves, stems	• Yellowish spots and deformation • Loss of vigor and leaves • Death of plant	• Spraying mineral oils	Rapisarda, 1985; Longo, 1996; Bayhan et al., 2006; Peri et al., 2006
	****: Pieris brassicae L. Pieris rapae (L.) Colotis evagore Lucas Anaphaeis aurota F. Colotis fausta fausta Olivier Lepidoptera Colotis liagore Klug. Cydia capparidana (Zel.) Lampides boeticus L.	Leaves, buds	• Holes in leaves • Deformation and abortion of buds	• Insecticides based on _Bacillus thuringiensis_	Pittaway, 1979; Murzin, 1986; Fernández Garica, 1988; Jordano Barbudo et al., 1988; Kontaxis, 1990; Longo, 1996; Bayhan et al., 2006; Peri et al., 2006
	*****: Capparimyia savastani (Martelli) Asphondylia gennadii (Marchal) Capparimyia savastani(Martelli)	Buds, fruits	Deformation and abortion	• "lur and kill" strategy with pyrethroids • Cultural practices	Harris, 1975; Orphanides, 1975; Rangarajan and Mahadewan, 1975; Donati and Belcari, 2003; Bayhan et al., 2006; Peri et al., 2006

*Cloeptera, **Heteroptera, ***Homoptera, ****Lepidoptera, *****Diptera, –, not available.

TABLE 3 | Chemical composition of the extracts from different organs of *Capparis spinosa* L.

Organs	Extracts	Chemical constituents	Location	References
Fruits	ME	(6S)-hydroxy-3-oxo-α-ionol glucoside,Corchoionoside C, prenyl glucoside, indol-3-acetonitrile glycoside, capparilloside A, capparilloside B.	Turkey	Calis et al., 2002
	AE	Flazin, guanosine, capparine A, capparine B, 1-H-Indole-3-carboxaldehyde, 4-hydroxy-1H-indole-3-carboxaldehyde, chrysoeriol, apigenin, kaempferol, thevetiaflavone, 5-hydroxymethylfuraldehyde, vanillic acid, cinnamic acid.	China	Haifeng et al., 2010
	EE/AF	Cappariside, 5-hydroxymethylfurfural, 5-hydroxymethyl furoic acid, 2-furoic acid.	China	Yang et al., 2010a
	EE/EF	Protocatechuic aldehyde, E-butenedioic acid, ethyl 3,4-dihydroxybenzoate, syringic acid, protocatechuic acid, vanillic acid, succinic acid, 4-hydroxybenzoic acid.		
	EE/AF	Capparisine A, capparisine B, capparisine C, 2-(5-hydroxymethyl-2-formylpyrrol-1-yl) propionic acid lactone, N-(30-maleimidy1)-5-hydroxymethyl-2-pyrrole formaldehyde.	China	Yang et al., 2010b
	EE	p-hydroxy benzoic acid, 5-(hydroxymethyl)furfural, bis(5-formylfurfural)ether, daucosterol, α-D-fructofuranosides methyl, uracil, stachydrine.	China	Feng et al., 2011
	EE/BF	Tetrahydroquinoline acid.	China	Zhang et al., 2014
	EE/EF	Racemic benzofuranone.		
	ME	Phenolics, flavonoids, carotenoids.	Bahrain	Allaith, 2014
Aerial Parts	ME	Quercetin 3-O-rutinoside, quercetin 3-O-glucoside, quercetin 3-O-glucoside-7-O-rhamnoside, Quercetin 3-O-[6‴ -α-L-rhamnosyl-6″ -β-D-glucosyl]-β-D-glucoside.	Egypt	Sharaf et al., 2000
	EE/HF	Terpene.	Jordan	Yang et al., 2010b; Muhaidat et al., 2013
	EE/AMF	Terpene, flavonoids.		
	EE/BF	Tanins, flavonoids, alkaloids.		
	EE/AF	Reducing sugar, flavonoids.		
Shoots and buds		Glucocapperin, glucoiberin, progoitrin, epiprogoitrin, sinigrin, gluconapoleiferin, glucoalyssin, gluconapin, 4-hydroxyglucobrassacin, glucobrassicanapin, glucobrassicin, gluconasturtiin.	Turkey	Matthäus and Ozcan, 2002
	EE/AMF	1-tetradecanol, methyl hexadecanoate, octadecanoic acid, 6,10,14-trimethyl-2-pentadecanone, β-sitosterol, glycerol monotetracostanoate, p-hydroxybenzaldehyde, ursolic acid, β-sitosterylglucoside, β-sitosterylglucoside-6′-octadecanoate.	Jordan	Khanfar et al., 2003
	EE/BF	4-coumaric acid, nicotinamide, cadabicine, isorhamnitine-3-O-rutinoside, rutin, stachydrine, 3-methyl-2-butenyl-β-glucoside.		
Leaves and Stems	EE	kaempferol 3-Rha-7-G, quercetin 3-Rut, quercetin 7-Rut, quercetin 3-G-7-Rhaw1.	China	Sharaf et al., 1997
Leaves and Flower buds	AE	5-Caffeoyl quinic acid, 1-Caffeoyl quinic acid, 5-p-Coumaroyl quinic acid, 4-Feruloyl quinic acid, Rutin, Quercetin 3-O-glc, Kaempferol 3-O-rutinoside, Methyl-quercetin-O-rutinoside, Kaempferol 3-O-glucoside, acids, flavonols.	Croatia	Kulisic-Bilusic et al., 2010
Roots	EE	Capparispine, Capparispine 26-O-b-D-glucoside, Cadabicine 26-O-b-D-glucoside hydrochloride.	China	Fu et al., 2008

ME, Methanolic Extraction; AE, Aqueous Extraction; EE, Ethanolic Extraction; AF, Aqueous Fraction; EF, Ethyl acetateFraction; BF, Butanol Fraction; HF, Hexane Fraction; AMF, Aqueous Methanol Fraction.

427.27 mg GAE/g DW of total phenolics was quantified in hydroethanolic extract of leaves. Iranian roots and fruits aqueous extracts contained 15.4 and 17.2 mg GAE/g DW respectively, lower than root ethyl acetate extracts containing 37.2 mg GAE/g DW and fruit ethanol extract containing 34.2 mg GAE/g DW.

Total flavonoids registered 57 mg Quercetin Equivalent (QE)/g DW in hydroethanol extract of leaves and ranged from 2.6 to 6.96 mg QE /g DW in leaves methanol extract, whereas, 13.97 mg QE/ g DW and 25 mg QE/ g DW were found in leaves and flowers aqueous extracts respectively. Roots and fruits ethyl acetate extracts had a content of flavonoids of 95.5 and 18.1 mg QE/g respectively (Bhoyar et al., 2011; Mahboubi and Mahboubi, 2014; Akkari et al., 2016; Mansour et al., 2016). According to Inocencio et al. (2000), 10 g of commercial caper bud will provide 40 mg QE as aglycone in Mediterranean countries (Spain, Turkey, Morocco, Italy, Greece). *C. spinosa* is cited as a very good source of phenolic acids, alkaloids, flavonoids (rutin, quercetin, kaempferol) and glucosinolates (glucocapparin, glucoiberin, sinigrin, glucobrassicin) (Sozzi and Vicente, 2006; Kulisic-Bilusic et al., 2012; Francesca et al., 2016). The latter having hydrolysis products known as anti-cancer agents (Mithen et al., 2000).

The glucosinolate content of caper parts varies between 84 and 89%. Young shoots contain the highest amount of glucosinolate whereas the content in buds decreased as their size decreased. Glucocapperin (methyl glucosinolate) is the main glucosinolate of shoots and buds whereas indole glucosinolate (4-hydroxyglucobrassicin) is present in trace amounts in leaves and shoots (2.04 μmol/g), glucocapparin and glucocleomin appeared in seeds and leaves (Matthäus and Ozcan, 2002). Seeds are rich in oils, proteins, and fibers. Seed oils are adapted for feed and have a high content of linoleic, and oleic acids, sterols (namely, sitosterol, campesterol, stigmasterol and Δ^5-avenasterol) and tocopherols (Akgül and Özcan, 1999; Matthäus and Özcan, 2005). In addition, the aliphatic (octadecanol as the major compound) and triterpenic (citrostadienol as the major compound) alcohol in the lipid unsaponifiable fraction were detected (Tlili et al., 2011b). These compounds can be integrated in cosmetic and pharmaceutical solutions. Seeds are rich in oils, proteins, and fibers. Seed oils are adapted for feed and food with a high content of linoleic, and oleic acids, sterols (namely, sitosterol, campesterol, stigmasterol and Δ^5-avenasterol) and tocopherols (Akgül and Özcan, 1999; Matthäus and Özcan, 2005). In addition, the aliphatic (octadecanol as the major compound) and triterpenic (citrostadienol as the major compound) alcohol in the lipid unsaponifiable fraction were detected (Tlili et al., 2011b). These compounds can be integrated in cosmetic and pharmaceutical solutions.

The fruit constituents have been subject of interest in several studies in order to determine the biochemical content which is of great benefit in biology and food industries. From fruits of *C. spinosa,* 11 organic acid compounds and a new antioxidant active compound were isolated and identified and the structures of five novel alkaloids were determined (Yang et al., 2010a,b). Carotenoids and some terpenoids such as tocopherol stabilize and photo-protect the lipid–phase of the cell membrane providing great tolerance to increased temperatures (Velikova et al., 2005; Camejo et al., 2006). Aqueous ethanolic fruit extracts contained flavonoids equivalent to rutin, phenolic compounds, tocopherol, carotenoid and vitamin C (Huseini et al., 2013). In addition to the known cappariloside A and stachydrine, an adenosine nucleoside, hypoxanthine and uracil were isolated from *C. spinosa* (Capparidaceae) fruits in China (Fu et al., 2007).

Essential Oils

The chemical composition of *C. spinosa* essential oils was subject to few studies (**Table 4**). Afsharypuor et al. (1998) determined 22 components in essential oil extracted from leaves, fruits and roots. The yield ranged from 0.02 to 0.9%. Fourteen components constituted leaf oil were detected (accounting for 91% of the total leaf oil composition); thymol (26.4%), isopropyl isothiocyanate (11%), 2-hexenal (10.2%), and butyl isothiocyanate (6.3%) represented the four major components. In the fruit oil only four components were detected (accounting for 98.5% of the total fruit oil composition); methyl isothiocyanate (41.6%) and isopropyl isothiocyanate (52.2%) were found as the two major components. Root components were represented mainly by methyl isothiocyanate (53.5%) and isopropyl isothiocyanate (31.4%). In Croatia, the essential oil of *C. spinosa* revealed that methyl isothiocyanate (92.06%) is the major compound in leaf and flower bud oils, in addition to benzyl isothiocyanate (0.74%), benzeneacetonitrile (0.40%), sec-butyl isothiocyanate (0.25%), and butyl isothioyanate (0.38%) (Kulisic-Bilusic et al., 2010). In the Eolian Archipelago, isothiocyanate is also a major component in caper, followed by benzyl isothiocyanate (Romeo et al., 2007). As to the Jordanian *C. spinosa*, essential oil is mostly represented by isopropyl isothiocyanate (28.92%), methyl isothiocyanate (25.6), and butyl isothiocyanate (16.65%) as major components (Muhaidat et al., 2013). Therefore, methyl isothiocyanate and isopropyl isothiocyanate are mainly present in fruits and roots, butyl-isothiocyanate is tissue-specific and is present in leaves but not in fruits and roots. Thiocyanate and isothiocyanate are break down products of glucosinolate as methyl glucosinolate (Glucocapperin) catalyzed by the enzyme myrosinase passes by the intermediate thiohydroximate and a rearrangement of the latter gives methyl isothiocyanate (Sozzi and Vicente, 2006).

Biological Activities

Several researchers have reported different biological activities of *C. spinosa extracts* in various *in vivo* and *in vitro* test models. Certain pharmacological properties of great interest of *C. spinosa* had been identified and others are being studied (Moufid et al., 2015). It is worth noting that most of the evidences about biological activity and phytochemistry still derive from the analysis of wild plant material.

C. spinosa aqueous extracts displayed a significant anti-hyperglycemic activity and anti-obesity effects (Eddouks et al., 2004, 2005; Lemhadri et al., 2007). Indeed, consumption of caper fruit extracts by diabetic rats induced a decrease in both blood sugars and blood triglycerides (Rahmani et al., 2013). Likewise, a study on caper fruit ethanol extracts on type 2 diabetic patients in Iran showed significant decrease in fasting blood glucose levels and glycosylated hemoglobin and also a significant decrease in triglyceride level, thus assuring previous results on the anti-hyperglycemic and hypolipidemic effects of *C. spinosa* (Huseini et al., 2013). In multi-low dose streptozotocin-induced (MLDS) diabetic mice, a treatment with aqueous extract from fruits of *C. spinosa* promotes insulin sensitivity in peripheral tissues resulting in a lower endogenous glucose production (EGP) in treated than in untreated mice (Eddouks et al., 2017). Both leaf and root ethanolic extracts of *C. spinosa* showed inhibition of pancreatic α-amylase activities that could be involved in the control of blood sugar (Selfayan and Namjooyan, 2016).

Additionally Aghel et al. (2010) showed that the ethanolic root bark extract of *C. spinosa* has a significant dose-dependent protection against carbon tetrachloride and induced liver damage *in vivo,* in accordance with Gadgoli and Mishra (1999), who previously found that *p*-Methoxy benzoic acid isolated from the methanol soluble fraction of the aqueous extract of *C. spinosa* L. aerial part possesses an antihepatotoxic activity. Using Swiss albino mice intoxicated with trichloroacetic acid (TCA), a synergic effect between a mixture of C. spinosa leaves and honey to cope with the TCA hepatotoxicity has been shown (Alzergy et al., 2015). Cisplatin is one of the premium-choice drugs for the treatment of many cancers but it is not without drawbacks,

TABLE 4 | Chemical composition of the essential oil of *Capparis spinosa* L. obtained by hydrodistillation.

References	Kulisic-Bilusic et al., 2010	Afsharypuor et al., 1998			Muhaidat et al., 2013
Collection location	Croatia	Iran			Jordan
Collection time	Jun-06	Aug-95			May-12
Species	*C. spinosa* L.	*C. spinosa* L. var. *mucronifolia* (Boiss.)			*C. spinosa* L. var. *aravensis*
Type of plant material	Floral buds and Leaves	Leaves	Fruits	Roots	Aerial parts
Yield	0.044% (w/w)	0.08% (v/w)	0.9% (v/w)	0.02% (v/w)	0.067% (w/w)
COMPONENT %					
Methyl isothiocyanate	92.06		41.6	53.5	25.6
Sec-butyl isothiocyanate	0.25		2.2	0.6	
Butyl isothiocyanate	0.38	6.3			16.65
2-butenyl isothiocyanate					2.24
Benzene acetaldehyde	0.23				
Benzene acetonitrile	0.4				
(E) β-Ionone	0.5				
Methyl methylsalicilate	0.17				
Benzyl isothiocyanate	0.74				
3-Hexenyl benzoate	0.27				
3-Hexenyl benzoate	1.75				
Isopropyl isothiocyanate		11	52.2	31.4	28.92
2-Hexenal		10.2			
Unknown		4.4	2.5	10.1	
γ-Terpinene		4.7			
n-Dodecane		1.8			
Carvone		2.3			
Thymol		26.4			
n-Tetradecane		4.3			
Geranyl acetone		3.5			
n-Hexadecane		5.5			
Dill apiole		2.4			
Palmitic acid		4.7			
n-Eicosane		3.5			
3-p-menthene					3.08
3-methylthio-1-hexanal					2.03
Total %	96.75	91	98.5	95.6	

principally toxicity to the liver and kidney. A recent work reported that methanolic extract of *C. spinosa* leaves significantly restored both the kidney and liver damages induced by cisplatin-treatment (Tlili et al., 2017).

Moreover, an *in vivo* study on murine indicated that the Tunisian *C. spinosa* leaf ethanol extract can stimulate melanogenesis in a dose-dependent manner without cytotoxicity. It can be useful in tanning and treating hair depigmentation (Matsuyama et al., 2009).

On the other hand, Panico et al. (2005) revealed the action exhibited by lyophilized extracts of *C. spinosa* (LECS) flower buds in developing novel anti-inflammatory/anti-degenerative agents that block the cartilage destruction during the inflammation *in vivo* and protect chondrocytes. More specifically, a recent study exhibited a better anti-inflammatory and analgesic effects for the fruits and stem-leaves of *C. spinosa* than those of the roots (Haifeng et al., 2010; Hong-Juan et al., 2014). The aqueous extracts from *C. spinosa* fruits were characterized as the best anti-inflammatory active fraction and also shown an anti-arthritic activity (Feng et al., 2011; Jiang et al., 2015).

Anti-inflammatory response of human peripheral blood mononuclear cells (PBMCs) induced by *C. spinosa* leaf extracts results from the control of cytokine gene expression (El Azhary et al., 2017). Cytokines constitute a category of small proteins (∼5–20 kDa) that are important in cell signaling and inflammatory response. On PBMCs, *C. spinosa* extracts are able to suppress the expression of *IL-17*, coding for a pro-inflammatory cytokine, and promote the expression of *IL-4*, coding for an anti-inflammatory cytokine. Kulisic-Bilusic et al. (2010) isolated the essential oil of *C. spinosa* flower buds and leaves and proved its antioxidant activity by β-carotene bleaching method and thiobarbituric acid reactive species essay. Moreover, this same essential oil and the aqueous infusion of the same plant parts showed anti-proliferative activity on colon cancer cells by decreasing the activation of nuclear factor NF-Kappa B (aqueous infusion had more inhibition activity than essential oil) and arresting the cell cycle at G2/M phase (Kulisic-Bilusic et al., 2012).

Great anti-oxidant activity was demonstrated also in fresh caper berries methanolic extracts; more specifically, fruit on liver hepatocellular carcinoma cells (HepG2) (Yu et al., 2017). Pain associated with rheumatoid arthritis and osteoarthritis was soothed after single dose administration of caper root decoction and hydroalcoholic extracts to rat models (Maresca et al., 2016). The latter extract explored cardio protective effect by reducing the undesired apoptotic effect of an anti-cancer drug, doxorubicin (Mousavi et al., 2016).

Moreover, anti-fungal activity against *Valsa mali* and inhibition of HIV-1 reverse transcriptase activities were shown with a 38 kDa protein purified from *C. spinosa* fresh seeds from fruits. Inhibition of colon cancer MT29 cells, breast cancer MCF-7 cells and hepatoma HepG2 proliferation was also attributed to this caper protein (Lam and Ng, 2009; Lam et al., 2009).

Aqueous caper bud extracts alleviated neurodegeneration induced by lipopolysaccharide in rats thus showed protective effect against cognitive diseases, learning, and memory damage (Goel et al., 2016). The decoction of *C. spinosa* roots showed significant inhibition activity on the growth of *Deinococcus radiophilus* (Boga et al., 2011). The butanolic and aqueous methanolic extract fractions from Jordanian *C. spinosa* showed antibacterial activity against *Staphylococcus epidermis* (ATCC 12228), whereas petroleum ether, hexane and water fractions exhibited antibacterial activity against *Streptococcus faecalis* (ATCC 29212) (Muhaidat et al., 2013).

TRADITIONAL USES

Food and Culinary

Caper is a potential source of valuable nutrients, since 100 g of caper fruit contain 67 mg calcium, 65 mg phosphorus, 9 mg iron, and 24.5 g protein.

Commercial capers are immature flower buds that can be pickled in salt or vinegar and used as an appetizer or condiment (Saadaoui et al., 2011). Hence, capers are included in hundreds of recipes due to their sharp piquant flavor owed to a complex organoleptic profile (Brevard et al., 1992) and are used as a seasoning to add pungency to sauces (e.g., tartare, remoulade, ravigote etc.,) dressings and salads (e.g., caponata, a cold eggplant salad with olives and capers), cold dishes and sauces served with salmon, herring, pasta and pizzas, cheeses, lamb, mutton, pork and chicken preparations (Sozzi and Vicente, 2006).

Unripe fruits called caper berries are also pickled and used as spices and condiments (Rivera et al., 2003). Food industries also use extracts from Caper buds and ripened fruits as flavor agents (Aliyazicioglu et al., 2015).

Medicinal

Capparis spinosa L. (Capparidaceae) is one of the medicinal plants that have been widely used in the traditional medicine during successive civilizations to cure various health disorders and illnesses. A wide range of therapeutic benefits are credited to caper extracts such as anti-hypertensive (Ali et al., 2007), anti-hepatotoxic (El Tanbouly et al., 1989; Gadgoli and Mishra, 1999), anti-diabetic (Kazemian et al., 2015; Mollica et al., 2017; Vahid et al., 2017), anti-obesity (Lemhadri et al., 2007), bronchorelaxant (Benzidane et al., 2013), anti-allergic and anti-histaminic (Angelini et al., 1991; Trombetta et al., 2005), anti-inflammatory (Al-Said et al., 1988; Zhou et al., 2010) or anti-biotic (Abraham et al., 2011; Mahboubi and Mahboubi, 2014) properties.

Iranian people used the root, fruit and plant bark of *C. spinosa* as diuretics and tonics against malaria and joint disease (Hooper, 1937; Afsharypuor et al., 1998). In Pakistan, leaves of *C. spinosa* are used as analgesic, anti-hemorrhoid, anti-rheumatic, aperients, deobstruent, depurative and diuretic (Chopra et al., 1986). In India, buds and roots of *C. spinosa* are useful in the treatment of boils, while leaves are used as counter-irritant and as a cataplasm in swellings. Roots are used to treat fever, rheumatism, paralysis, toothache and kill worms in the ear. Bark is used against coughs, asthma and inflammation (Wealth of India, 1992). The stem-leaves, fruits, and roots have been used for the treatment of rheumatoid arthritis and gout in traditional medicine in China (Feng et al., 2011).

The root bark of Caper has been used as an analgesic and carminative agent and possesses antihypertensive activity as well (Eddouks et al., 2004; Lemhadri et al., 2007). Decoctions from root bark are also used to treat dropsy, anemia and rheumatism. Herbal tea made from root and young shoots is of benefit for the treatment of rheumatism, stomach and intestinal disorders. In the folklore of the central region of Saudi Arabia, along with *C. spinosa* diuretic and body tonic utilization, pastes prepared from the root bark are used externally to treat swollen joints, skin rashes and dry skin. During the last decade, some cosmetic products derived from *C. spinosa* fruit extract (e.g., Gatuline® Derma-Sensitive; SKIN MOON®; SKIN SAVE®) were commercialized, claiming skin protection and anti-aging or anti-inflammatory properties. Herbal tea prepared by *C. spinosa* buds and leaves is found to be a popular remedy against cold and related infections, also decoction of buds and leaves is used internally for curing gastrointestinal infections, diarrhea, and dysentery and also useful for the removal of kidney stones (Sher and Alyemeni, 2010). In Morocco, unopened buds are used externally to treat eye infections and prevent cataracts while caper dried fruits are meant to cure hypertension and diabetic complications when taken orally with a glass of water (Jouad et al., 2001; Eddouks et al., 2002).

Recent review articles provide a detailed overview of the state of the art in the field of medicinal/pharmaceutical properties of *C. spinosa* (Moufid et al., 2015; Anwar et al., 2016; Nabavi et al., 2016; Rahnavard and Razavi, 2016).

CONCLUSION

This review encourages further studies on *C. spinosa* in the East Mediterranean countries to face the changing environment, climate-mediated transition of agriculture and to promote its nutritional and health benefits. This plant has various medicinal, culinary, agronomic and economic values. Caper cultivation could be a good solution for implementing needed novel agricultural practices for climate risk management and production sustainability. Its remarkable ability to adapt to different climates call upon to integrate *C. spinosa* in the long-term agricultural strategy to cope with larger impacts of climate changes in the future.

The establishment of genetic data for taxonomic identification and productivity is a priority research need for caper. Genetic variability for tolerance to heat stress should be exploited in order to screen germplasm and select cultivars with high temperature tolerant genotypes. High genetic potential can also be exhibited by selection of hybrids and induced crossings.

The identification of molecular markers correlated with phenotypic traits of caper will be a future tool to promote stress-related breeding programs, as well as an integrative view of the biology of the species and its evolution.

The traditional medicinal knowledge and the biological studies have to find ways to enlarge the benefits and the capacities of this natural plant resource.

Finally, this plant could be integral part of family farming and value chain products in the Mediterranean contributing enormously to socio-economic development.

AUTHOR CONTRIBUTIONS

SC, wrote the paper. AA, ME, LC, NO, and LR made some corrections and additional contributions.

FUNDING

SC fellowship was funded by the "Women's association" in Hadath el Jebbeh, Lebanon.

ACKNOWLEDGMENTS

To the Saclay Plant Sciences (SPS) LabEx supporting Jean-Pierre Bourgin Institute (ANR-10-LABX-0040-SPS).

REFERENCES

Abraham, S. V. P. I., Palani, A., Ramaswamy, B. R., Shunmugiah, K. P., and Arumugam, V. R. (2011). Antiquorum sensing and antibiofilm potential of *Capparis spinosa*. *Arch. Med. Res.* 42, 658–668. doi: 10.1016/j.arcmed.2011.12.002

Adams, M. J., Antoniw, J. F., Bar-Joseph, M., Brunt, A. A., Candresse, T., Foster, G. D., et al. (2004). The new plant virus family *Flexiviridae* and assessment of molecular criteria for species demarcation. *Arch. Virol.* 149, 1045–1060. doi: 10.1007/s00705-004-0384-x

Afsharypuor, S., Jeiran, K., and Jazy, A. A. (1998). First investigation of the flavor profiles of leaf, ripe fruit and root of *Capparis spinosa* var. mucronifolia from Iran. *Pharm. Acta. Helv.* 5, 307–309. doi: 10.1016/S0031-6865(97)00023-X

Aghel, N., Rashidi, I., and Mombeini, A. (2010). Hepatoprotective activity of *Capparis spinosa* root bark against CCl4 induced hepatic damage in mice. *Iran. J. Pharm. Res.* 6, 285–290.

Ahmed, M. (1986). Vegetation of some foothills of Himalayan range in Pakistan. *Pak. J. Bot.* 18, 261–269.

Akgül, A., and Özcan, M. (1999). Some compositional characteristics of Capers (*Capparis* spp.) seed and oil. *Grasas Aceites*. 50, 49–52. doi: 10.3989/gya.1999.v50.i1.635

Ali, Z. N., Eddouks, M., and Michael, J. B. (2007). Cardiovascular effect of *Capparis spinosa* aqueous extract. Part III: antihypertensive effect in spontaneously hypertensive rats. *Am. J. Pharmacol. Toxicol.* 2, 111–115. doi: 10.3844/ajptsp.2007.111.115

Akkari, H., B'chir, F., Hajaji, S., Rekiki, M., Sebai, E., Hamza, H., et al. (2016). Potential anthelmintic effect of *Capparis spinosa* (Capparidaceae) as related to its polyphenolic content and antioxidant activity. *Vet. Med.* 61, 308–316. doi: 10.17221/169/2015-VETMED

Aliyazicioglu, R., Tosun, G., and Eyupoglu, E. (2015). Characterisation of volatile compounds by spme and gc-fid/ms of capers (*Capparis spinosa* L.). *Afr. J. Agric. Res.* 10, 2213–2217.

Alkire, B. (1998). *Capers. Center for New Crops and Plants Products.* West Lafayette, IN: Purdue University.

Allaith, A. A. A. (2014). Assessment of the antioxidant properties of the caper fruit (*Capparis spinosa* L.) from Bahrain. *J. Assoc of Arab Univ. Basic Appl. Sci.* 19, 1–7. doi: 10.1016/j.jaubas.2014.07.001

Al-Safadi, B., and Elias, R. (2011). Improvement of caper (*Capparis spinosa* L.) propagation using *in vitro* culture and gamma irradiation. *Sci. Hortic.* 127, 290–297. doi: 10.1016/j.scienta.2010.10.014

Al-Safadi, B., Faouri, H., and Elias, R. (2014). Genetic diversity of some *Capparis* L. species growing in Syria. Braz. *Arch. Biol. Technol.* 57, 916–926. doi: 10.1590/S1516-8913201402549

Al-Said, M. S., Abdelsattar, E. A., Khalifa, S. I., and El-Feraly, F. S. (1988). Isolation and identification of an anti-inflammatory principle from *Capparis spinosa*. *Die Pharmazie* 43, 640–641.

Al-Yemeni, M. N., and Zayed, K. M. (1999). Ecology of some plant communities along Riyadh Al-Thumamah Road, Saudi Arabia. Saudi. *J. Biol. Sci.* 6, 9–26.

Alzergy, A. A., Elgharbawy, S. M., Mahmoud, G. S., and Mahmoud, M. R. (2015). Role of *Capparis spinosa* in ameliorating trichloroacetic acid induced toxicity in liver of Swiss albino mice. *Life Sci. J.* 12, 26–39.

Andrade, G., Esteban, E., Velasco, L., Lorite, M. J., and Bedmar, E. J. (1997). Isolation and identification of N2-fixing microorganisms from the rhizosphere of *Capparis spinosa* (L.). *Plant Soil.* 197, 19–23. doi: 10.1023/A:1004211909641

Angelini, G., Vena, G. A., Filotico, R., Foti, C., and Grandolfo, M. (1991). Allergic contact dermatitis from *Capparis spinosa* L. applied as wet compresses. *Contact Derm.* 24, 382–383. doi: 10.1111/j.1600-0536.1991.tb01764.x

Anwar, F., Muhammad, G., Hussain, M. A., Zengin, G., Alkharfy, K. M., Ashraf, M., et al. (2016). *Capparis spinosa* L.: a plant with high potential for development of functional foods and nutraceuticals/pharmaceuticals. *Int. J. Pharm.* 12, 201–219. doi: 10.3923/ijp.2016.201.219

Bahrani, M. J., Ramazani, G. M., Shekafandeh, A., and Taghvaei, M. (2008). Seed germination of wild Caper (*Capparis spinosa* L., var. parviflora) as affected by dormancy breaking treatments and salinity levels. *Seed Sci. Technol.* 36, 776–780. doi: 10.15258/sst.2008.36.3.27

Barbera, G. (1991). "Le câprier (*Capparis* spp.)," in *Programme de Recherche Agrimed*, ed B. Guiseppe (Luxembourg: Commission des Communautés européennes L- 2920), 62.

Barbera, G., and Di Lorenzo, R. (1984). The caper culture in Italy. *Acta Hortic.* 144, 167–171. doi: 10.17660/ActaHortic.1984.144.21

Bayhan, E., Ulusoy, M. R., and Brown, J. K. (2006). Host range, distribution, and natural enemies of Bemisia tabaci 'B biotype' (Hemiptera: Aleyrodidae) in Turkey. *J. Pest. Sci.* 79, 233–240. doi: 10.1007/s10340-006-0139-4

Benzidane, N., Charef, N., Krache, I., Baghiani, A., and Arrar, L. (2013). *In vitro* bronchorelaxant effects of *Capparis Spinosa* aqueous extracts on rat trachea. *J. Appl. Pharm. Sci.* 3, 85–88

Bhoyar, M. S., Mishra, P. G., Naik, K. P., Murkute, A. A., and Srivastavar, B. R. (2012). Genetic variability studies among natural populations of *Capparis spinosa* from cold arid desert of trans-himalayas using DNA markers. *Natl. Acad. Sci. Lett.* 35, 505–515. doi: 10.1007/s40009-012-0086-y

Bhoyar, M. S., Mishra, P. G., Naik, K. P., and Srivastava, R. (2011). Estimation of antioxidantactivity and total phenolics among natural populations of Caper (*Capparis spinosa*) leaves collected from cold arid desert of trans-Himalayas. *Aust. J. Crop. Sci.* 5, 912–919.

Bita, C. E., and Gerats, T. (2013). Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Front. Plant Sci.* 4:273. doi: 10.3389/fpls.2013.00273

Boga, C., Forlani, L., Calienni, R., Hindley, T., Hochkoeppler, A., Tozzi, S., et al. (2011). On the antibacterial activity of roots of *Capparis spinosa* L. *Nat. Prod. Res.* 25, 417–421. doi: 10.1080/14786419.2010.487189

Bond, R. E. (1990). The caper bush. *Herbarist* 56, 77–85.

Bounous, G., and Barone, E. (1989). Il cappero: prospettive di sviluppo di specie legnose per le zone aride e semi-aride del meridione e nuovi criteri di utilizzo. *Terra e Sole*. 44, 733–735.

Brevard, H., Brambilla, M., Chaintreau, A., Marion, J. P., and Diserens, H. (1992). Occurrence of elemental sulphur in capers (*Capparis spinosa* L.) and first investigation of the flavour profile. *Flav. Frag. J.* 7, 313–321. doi: 10.1002/ffj.2730070605

Caglar, C., Caglar, S., Ergin, O., and Yarim, M. (2005). The influence of growth regulators on shoot proliferation and rooting of *in vitro* propagated caper. *J. Environ. Biol.* 26, 479–485.

Caliş, I., Kuruüzüm, A., Lorenzetto, P. A., and Rüedi, P. (2002). (6S)-Hydroxy-3-oxo-a-ionol glucosides from *Capparis spinosa* fruits. *Phytochemistry* 59, 451–457. doi: 10.1016/S0031-9422(01)00399-5

Camejo, D., Jiménez, A., Alarcón, J. J., Torres, W., Gómez, J. M., and Sevilla, F. (2006). Changes in photosynthetic parameters and antioxidant activitis following heat-shock treatment in tomato plants. *Funct. Plant Biol.* 33, 177–187. doi: 10.1071/FP05067

Carra, A., Del Signore, M. B., Sottile, F., Ricci, A., and Carimi, F. (2011). Potential use of new diphenylurea derivatives in micropropagation of *Capparis spinosa* L. *Plant Growth Regul.* 66, 229–237. doi: 10.1007/s10725-011-9645-3

Carra, A., Sajeva, M., Abbate, L., Siragusa, M., Sottile, F., and Carimi, F. (2012). *In vitro* plant regeneration of caper (*Capparis spinosa* L.) from floral explants and genetic stability of regenerants. *Plant Cell Tissue Organ Cult.* 109, 373–381. doi: 10.1007/s11240-011-0102-9

Chalak, L., and Elbitar, A. (2006). Micropropagation of *Capparis spinosa* L. subsp. *rupestris* sibth & Sm. by nodal cuttings. *Indian J. Biotechnol.*. 5, 555–558.

Chalak, L., Elbitar, A., Cordahi, N., Hage, C., and Chehade, A. (2003). *In vitro* propagation of *Capparis spinosa* L. *Acta Hortic*. 616, 335–338. doi: 10.17660/ActaHortic.2003.616.48

Chalak, L., Perin, A., Elbitar, A., and Chehade, A. (2007). Phenotypic diversity and morphological characerization of *Capparis spinosa* L. in Lebanon. *Biol. Tunisie* 4, 28–32.

Chopra, R. N., Nayar, S., and Chopra, I. C. (1986). *Glossary of Indian Medicinal Plants (with Supplement)*. New Delhi: Council of Scientific and Industrial Research.

Ciferri, R. (1949). Rassegna di parassiti e malattie del cappero (*Capparis spinosa* L.) in Italia. *Notiziario sulle Malattie delle Piante* 3, 33–35.

Colazza, S., Guarino, S., and Peri, E. (2004). *Bagrada hilaris* (Burmeister) (Heteroptera: Pentatomidae) fitofago dannoso al cappero nell'isola di Pantelleria. *Inf. Fitopatol.* 53, 30–34.

Danin, A. (2010). *Capparis* in the East Mediterranean countries. *Fl. Medit.* 20, 179–185.

Di Franco, A., and Gallitelli, D. (1985). Rhabdovirus-like particles in caper leaves with vein yellowing. *Phytopathol. Mediterr*. 24, 234–236.

Donati, M., and Belcari, A. (2003). A note on insect pests of the caper plant in Jordan, with special reference to *Capparimyia savastani* (Martelli) (Diptera, Tephriti-dae). *Stud. dipterol.* 10, 395–400.

Eddouks, M., Lemhadri, A., Hebi, M., Hidani, A. E., Zeggwagh, N. A., El Bouhali, B., et al. (2017). *Capparis spinosa* L. aqueous extract evokes antidiabetic effect in streptozotocin-induced diabetic mice. *Avicenna J. Phytomed*. 7, 191–198.

Eddouks, M., Lemhardi, A., and Michel, J. B. (2004). Caraway and caper potential antihyperglycaemic plants in diabetic rats. *J. Ethnopharmacol.* 94, 143–148. doi: 10.1016/j.jep.2004.05.006

Eddouks, M., Lemhardi, A., and Michel, J. B. (2005). Hypolipidemic activity of aqueous extract of *Capparis spinosa* L. in normal and diabetic rats. *J. Ethnopharmacol*. 98, 345–350. doi: 10.1016/j.jep.2005.01.053

Eddouks, M., Maghrani, M., Lemhadri, A., Ouahidi, M. L., and Jouad, H. (2002). Ethnopharmacological survey of medicinal plants used for the treatment of diabetes mellitus, hypertension and cardiac diseases in the south-east region of Morocco (Tafilalet). *J. Ethnopharmacol.* 103, 82–97. doi: 10.1016/S0378-8741(02)00164-2

El Azhary, K., Jouti, N. T., El Khachibi, M., Moutia, M., Tabyaoui, I., El Hou, A., et al. (2017). Anti-inflammatory potential of *Capparis spinosa* L. in vivo in mice through inhibition of cell infiltration and cytokine gene expression. *BMC Complement Altern. Med*. 17:81 doi: 10.1186/s12906-017-1569-7

El Tanbouly, N., Joyeux, M., Hanna, S., Fleurentin, J., El Alfy, T., and Anton, R. (1989). Antihepatotoxic effect of aqueous extracts from *Capparis spinosa*. *Planta Medica*. 55, 95–95. doi: 10.1055/s-2006-961847

Faran, M. (2014). "Capparis spinosa - the plant on the wall," in *Medicinal and Aromatic Plants of the Middle-East (Medicinal and Aromatic Plants of the World)*, eds Z. Yaniv and N. Dudai (Dordrecht: Springer Netherlands), 59–65.

Feng, X., Lu, J., Xin, H., Zhang, L., Wang, Y., and Tang, K. (2011). Anti-arithritic active fraction of *Capparis spinosa* L. fruits and its chemcial constituents. *Yakugaki Zasshi* 13, 423–429. doi: 10.1248/yakushi.131.423

Fernández Garica, E. (1988). Spring and summer hosts for *Pieris rapae* in Southern Spain with special attention to *Capparis spinosa*. *Entomol. Exp. Appl*. 48, 173–178. doi: 10.1111/j.1570-7458.1988.tb01161.x

Fici, S. (2001). Intraspecific variation and evolutionary trends in *Capparis spinosa* L. (Capparaceae). *Plant Syst. Evol.* 228, 123–141. doi: 10.1007/s006060170024

Fici, S. (2014). A taxonomic revision of the *Capparis spinosa* group (Capparaceae) from the Mediterranean to Central Asia. *Phytotaxa*. 174, 1–24. doi: 10.11646/phytotaxa.174.1.1

Fici, S. (2015). A taxonomic revision of the *Capparis spinosa* group (Capparaceae) from eastern Africa to Oceania. *Phytotaxa*. 203, 24–36. doi: 10.11646/phytotaxa.203.1.2

Francesca, N., Barbera, M., Martorana, A., Saiano, F., Gaglio, R., Aponte, R., et al. (2016). Optimised method for the analysis of phenolic compounds from caper (*Capparis spinosa* L.) berries and monitoring of their changes during fermentation. *Food Chem.* 196, 1172–1179 doi: 10.1016/j.foodchem.2015.10.045

Fu, X. P., Aisa, A. H., Abdurahim, M., Yili, A., Aripova, F. S., and Tashkhodzhaev, B. (2007). Chemical composition of *Capparis spinosa* fruit. *Chem. Nat. Compd.* 43, 181–183. doi: 10.1007/s10600-007-0074-5

Fu, X. P., Wu, T., Abdurahim, M., Su, Z., Hou, L. X., Aisia, A. H., et al. (2008). New spermidine alkaloids from *Capparis spinosa* roots. *Phytochem. Lett.* 1, 59–62. doi: 10.1016/j.phytol.2008.01.001

Gadgoli, C., and Mishra, S. H. (1999). Antihepatotoxic activity of *p*-methoxy benzoic acid from *Capparis spinosa. J. Ethnopharmacol.* 66, 187–192. doi: 10.1016/S0378-8741(98)00229-3

Gallitelli, D., and Di Franco, A. (1987). Characterization of caper latent virus. *J. Fitopatol*. 119, 97–105 doi: 10.1111/j.1439-0434.1987.tb00471.x

Gan, L., Zhang, C., Yin, Y., Lin, Z., Huang, Y., Xiang, J., et al. (2013). Anatomical adaptations of the xerophilous medicinal plant, *Capparis spinosa*, to drought conditions. *Hortic. Environ. Biotechnol*. 54, 156–161. doi: 10.1007/s13580-013-0162-3

Goel, A., Digvijaya, Garg, A., and Kumar, A. (2016). Effect of *Capparis spinosa* Linn. extract on lipopolysaccharide-induced conginitive impairments in rats. *Indian J. Exp. Biol*. 54, 126–132.

González, S. (1973). La alcaparra: características y comercialización. *Agricultura* 495, 422–425.

Gorini, F. (1981). Schede orticole. 6. Ortaggi da infiorescenze. 6.4. Cappero. *Informatore di Ortoflorofrutticoltura*. 6, 3–4.

Güleryüz, M., Özkan, G., and Ercisli, S. (2009). "Caper (*Capparis* spp.) growing techniques and economical importance," in *1st International Symposium on Sustainable Development* (Sarajevo), 94–97.

Gull, T., Anwar, F., Sultana, B., Alcayde, C. A. M., and Nouman, W. (2015). *Capparis* species: a potenial source of bioactives and high-value comonents: a review. *Ind. Crops Prod.* 67, 81–96. doi: 10.1016/j.indcrop.2014.12.059

Gupta, A. K., and Bhardwaj, L. N. (1998). Additional host of *Leveillula taurica (Lev.)* G. Arnaud from India. *Indian Phytopathol.* 51, 104–106.

Haifeng, Z., Renji, J., Jie, K., Xiaoling, H., Yan, L., Changlong, Z., et al. (2010). Antiinflammatory effects of caper *(Capparis spinosa L.)* fruit aqueous extract and the isolation of main phytochemicals. *J. Agric. Food Chem.* 58, 12717–12721. doi: 10.1021/jf1034114

Hall, C. J., Systma, J. K., and Iltis, H. H. (2002). Phylogeny of Capparaceae and Brassicaceae *based* on chloroplast seauence data. *Am. J. Bot.* 89, 1826–1842. doi: 10.3732/ajb.89.11.1826

Hansen, J. (1991). *The Palaeoethnobotany of Franchthi Cave*. Bloomington: Indiana University Press.

Harris, K. M. (1975). The taxonomic status of the carob gall midge, *Asphondylia gennadii* (Marchal), comb. *n.* (Diptera, Cecidomyiidae), and of other Asphondylia species recorded from Cyprus. *Bul. Entomol. Res.* 65, 377–380. doi: 10.1017/S0007485300006040

Hatfield, J. L., and Prueger, J. H. (2015). Temperature extremes: effect on plant growth and development. *Weather Clim. Extremes* 10, 4–10. doi: 10.1016/j.wace.2015.08.001

Heywood, V. H. (1964). *"Capparis* L.," in *Flora Europaea*, ed V. H. Heywood, T. G. Tutin, N. A. Bugres, D. M. Moore, D. H. Valentine, S. M. Waletrs, et al. (Cambridge Cambridge University Press), 259.

Higton, R. N., and Akeroyd, J. R. (1991). Variation in *Capparis spinosa* L. in Europe. *Bot. J. Linn. Soc.* 106, 104–112.

Hillman, G. C. (1989). "Late Palaeolithic plant foods from Wadi Kubbaniya in Upper Egypt: dietary diversity, infant weaning, and seasonality in a riverine environment," in *Foraging and Farming: The Evolution of Plant Exploitation*, ed D. R. Harris and G. C. Hillman (London: Unwin Hyman), 207–239.

Hong-Juan, L., Tao, Y., Xue-Mei, C., and Chang-Hong, W. (2014). Comparative evaluation of anti-inflammatory and analgesic activities of various medicinal parts of *Capparis spinosa*: a consideration of ecological environment and resource conservation. *Indian J. Med. Res. Pharm. Sci.* 4, 53–59.

Hooper, D. (1937). *Useful Plants and Drugs of Iran and Iraq*. Chicago: Field Museum of Natural History.

Howden, M. S., Soussana, J. F., Tubiello, N. F., Chhetri, N., Dunlop, M., and Holger Meinke, H. (2007). Adapting agriculture to climate change. *Proc. Natl. Acad. Sci. U.S.A.* 104, 19691–19696. doi: 10.1073/pnas.07018 90104

Huseini, F. H., Hasani-Rnjbar, S., Nayebi, N., Heshmat, R., Sigaroodi, K. F., Ahvazi, M., et al. (2013). *Capparis spinosa* L. (Caper) fruit extract in treatment of type 2 diabetic patients: a randomized double-blind placebo-controlled clinical trial. *Complement Ther. Med.* 21, 447–452. doi: 10.1016/j.ctim.2013. 07.003

Infantino, A., Pucci, N., Di Giambattista, G., and Tomassoli, L. (2006). *Capparis spinosa-* a new host for Sclerotium rolfsii. *Plant Pathol.* 55, 580. doi: 10.1111/j.1365-3059.2006.01376.x

Infantino, A., Tomassoli, L., Peri, E., and Colazza, S. (2007). Viruses, fungi and insect pests affecting caper. *Eur. J. Plant Sci. Biotech.* 1, 170–179.

Inocencio, C., Alcaraz, F., Calderon, F., Obon, C., and Rivera, D. (2002). The use of floral characters in *Capparis* sect. Capparis' to determine the botanical and geographical origin of capers. *Eur. Food Res. Technol.* 214, 335–339. doi: 10.1007/s00217-001-0465-y

Inocencio, C., Alcaraz, F., and Tomas-Barberan, F. (2000). Flavonoid content of commercial Capers (*Capparis spinosa, C. sicula* and *C. orientalis*) produced in Mediterranean countries. *Eur. Food Res. Technol.* 212, 70–74. doi: 10.1007/s002170000220

Inocencio, C., Cowan, S. R., Alcaraz, F., Rivera, D., and Fay, F. M. (2005). AFLP fingerprinting in *Capparis* subgenus *Capparis* related to the commercial sources of capers. *Genet. Resour. Crop. Evol.* 52, 137–144. doi: 10.1007/s10722-003-4432-2

Inocencio, C., Rivera, D., Obon, C., Alcaraz, F., and Barrena, J. A. (2006). A systematic revision of *Capparis* section *Capparis* (Capparaceae). *Ann. Missouri. Bot. Gard.* 93, 122–149. doi: 10.3417/0026-6493(2006)93[122:ASROCS]2.0. CO;2

Jacobs, M. (1960). Capparidaceae. *Flora Malesiana*. 1, 61–105.

Jacobs, M. (1965). The genus *Capparis* (Capparaceae) from the Indus to the Pacific. *Blumea* 12, 385–541.

Jiang, H. E., Li, X., Ferguson, K. D., Wang, Y. F., Liu, C. J., and Li, C. S. (2007). The discovery of *Capparis spinosa* L. (Capparidaceae) in the Yanghai Tombs (2800 years b.p.), NW China, and its medicinal implications. *J. Ethnopharmacol.* 113, 409–420. doi: 10.1016/j.jep.2007.06.020

Jiang, S. S., Ma, W. N., Lu, W. J., and Ma, G. Z. (2015). Preliminary screening of anti- inflammatory active fractions from fruits of *Capparis spinosa* of uighur medicine. *Chin. J. Expe. Trad. Med. Form.* 4:041.

Jordano Barbudo, D., Rodriguez Gonzalez, J., and Fernandez Haeger, J. (1988). *Capparis spinosa* (Capparidaceae): on oviposition substrate for *Lampides boeticus* Linnaeus, in southern Spain (Lepidoptera: Lycaenidae). *Nota Lepid.* 10, 218–223.

Jouad, H., Rhiouani, H., El Hilaly, J. M., and Eddouks, M. (2001). Ethnobotanical survey of medicinal plants used for the treatment of diabetes, cardiac and renal diseases in the North centre region of Morocco (Fez–Boulemane). *J. Ethnopharmacol.* 77, 175–182. doi: 10.1016/S0378-8741(01)00289-6

Kala, P. C., and Mathur, B. V. (2002). Patterns of plant species distribution in the Trans-Himalayan region of Ladakh, India. *J. Veget. Sci.* 13, 751–754. doi: 10.1111/j.1654-1103.2002.tb02104.x

Kavak, H. (2004). Epidemic outbreaks of powdery mildew caused by *Leveillula taurica* on *Capparis spinosa* in Turkey. *Plant Pathol.* 53:809. doi: 10.1111/j.1365-3059.2004.01072.x

Kazemian, M., Abad, M., Haeri, M. R., Ebrahimi, M., and Heidari, R. (2015). Anti-diabetic effect of *Capparis spinosa* L. root extract in diabetic rats. *Avicenna J. Phytomed.* 5:325.

Khanfar, M. A., Sabri, S. S., Zarga, M. H., and Zeller, K. P. (2003). The chemical constituents of *Capparis spinosa* of jordanian origin. *Nat. Prod. Res.* 17, 9–14. doi: 10.1080/10575630290034302

Khouildi, S., Pagnotta, M. A., Tanzarella, O. A., Ghorbel, A., and Porceddu, E. (2000). Suitability of RAPD (random amplified polymorphic DNA) technique for estimating the genetic variation in natural genotypes of Tunisian and Italian caper (*Capparis spinosa* L.). *Agricoltura-Mediterranea* 130, 72–77.

Kontaxis, D. G. (1990). Pest of caper, *Capparis spinosa*. Some new records for California. *Phytopathology*. 80:1026.

Kulisic-Bilusic, T., Balzevic, I., Dejanovic, B., Milos, M., and Pifat, G. (2010). Evaluation of the antioxidant activity of essential oils from caper (*Capparis spinosa*) and sea fennel (*Crithmum maritimum*) by different methods. *J. Food Biochem.* 34, 286–302. doi: 10.1111/j.1745-4514.2009.00330.x

Kulisic-Bilusic, T., Schmoller, I., Schnäbele, K., Siracusa, L., and Ruberto, G. (2012). The anticarcinogenic potential of essential oil and aqueous infusion from caper (Capparis spinosa L.). *Food Chem.* 132, 261–267. doi: 10.1016/j.foodchem.2011. 10.074

Lam, S. K., Han, Q. F., and Ng, T. B. (2009). Isolation and characterization of a lectin with potentially exploitable activities from caper (*Capparis spinosa*) seeds. *Biosci. Rep.* 29, 293–299. doi: 10.1042/BSR20080110

Lam, S. K., and Ng, T. B. (2009). A protein with antiproliferative, antifungal and HIV-1 reverse transcriptase inhibitory activities from caper (*Capparis spinosa*) seeds. *J. Phytomed.* 16, 444–450. doi: 10.1016/j.phymed.2008. 09.006

Legua, P., Martínez, J. J., Melgarejo, P., Martínez, R., and Hernández, F. (2013). Phenological growth stages of caper plant (*Capparis spinosa* L.) according to the Biologische Bundesanstalt, Bundessortenamt and CHemical scale. *Ann. Appl. Biol.* 163, 135–141. doi: 10.1111/aab.12041

Lemhadri, A., Eddouks, M., Sulpice, T., and Burcelin, R. (2007). Antihyperglycaemic and anti-obesity effects of *Capparis spinosa* and *Chamaemelum nobile* aqueous extracts in HFD Mice. *Am. J. Pharm. Toxicol.* 2, 106–110. doi: 10.3844/ajptsp.2007.106.110

Levizou, E., Drilias, P., and Kyparissis, A. (2004). Exceptional photosynthetic performance of *Capparis spinosa* L. under adverse conditions of Mediterranean summer. *Photosynthetica* 42, 229–235. doi: 10.1023/B:PHOT.0000040594.85407.f4

Li, Q., Yu, L., Deng, Y., Li, W., Li, M., and Cao, J. (2007). Leaf epidermal characters of *Lonicera japonica* and *Lonicera confusa* and their ecology adaptation. *J. For. Res.* 18, 103–108. doi: 10.1007/s11676-007-0020-1

Liotta, G. (1977). Acalles barbarus Lucas (s.l.) su Capparis spinosa L. a Pantelleria (Col. Curculionidae), Nota bio-etologica' (Summary in English). *Nat. Sicil* 1, 39–45.

Liu, C., Xue, P. G., Cheng, B., Wang, X., He, J., Liu, H. G., et al. (2015). Genetic diversity analysis of *Capparis spinosa* L. populations by using ISSR markers. *Genet. Mol. Res.* 14, 16476–16483. doi: 10.4238/2015.December.9.19

Longo, S. (1996). La mosca del cappero. *Inf. Agrar*. 52, 65–69.

Lorente, F. L., and Vicente, M. P. (1985). *La Tapenera o Alcaparra: Cultivo y Aprovechamiento*. Madrid: Ministerio de Agricultura, Pesca y Alimentación.

Mahboubi, M., and Mahboubi, A. (2014). Antimicrobial activity of *Capparis spinosa* as its usages in traditional medicine. *Herba Pol.* 60, 39–48. doi: 10.2478/hepo-2014-0004

Maire, R. (1965). Flore de l'Afrique du Nord. *Encycl. Biol.* 67, 256–302.

Mansour, B. R., Jilani, H. B. I., Bouaziz, M.,Gargouri, B., Elloumi, N., Attia, H., et al. (2016). Phenolic contents and antioxidant activity of ethanolic extract of *Capparis spinosa. Cytotechnology* 68, 135–142. doi: 10.1007/s10616-014-9764-6

Maresca, M., Micheli, L., Mannelli, L. D. C., Tenci, B., Innocenti, M., Khatib, M., et al. (2016). Acute effect of *Capparis spinosa* root extracts on rat articular pain. *J. Ethnopharmacol.* 193, 456–465. doi: 10.1016/j.jep.2016.09.032

Matsuyama, K., Villareal, M. O., Omri, A. E., Han, J., Kchouk, M. E., and Isoda, H. (2009). Effect of Tunisian *Capparis spinosa* L. extract on melanogenesis in B16 murine melanoma cells. *J. Nat. Med.* 63, 468–472. doi: 10.1007/s11418-009-0355-3

Matthäus, B., and Özcan, M. (2005). Glucosinolates and fatty acid, sterol, and tocopherol composition of seed oils from *Capparis spinosa* var. spinosa and Capparis ovata Desf. var. canescens (Coss.) *Heywood. J. Agric. Food. Chem.* 53, 7136–7141. doi: 10.1021/jf051019u

Matthäus, B., and Ozcan, M. (2002). Glucosinolate composition of young shoots and flower buds of capers (*Capparis* Species) growing wild in Turkey. *J. Agric. Food. Chem.* 50, 7323–7325. doi: 10.1021/jf020530+

Mithen, F. R., Dekker, M., Verkerk, R., Rabot, S., and Johnson, I. T. (2000). The nutritional significance, biosynthesis and bioavailability of glucosinolates in human food. *J. Sci. Food Agric.* 80, 967–984. doi: 10.1002/(SICI)1097-0010(20000515)80:7<967::AID-JSFA597>3.0.CO;2-V

Mollica, A., Zengin, G., Locatelli, M., Stefanucci, A., Mocan, A., Macedonio, G., et al. (2017). Anti-diabetic and anti-hyperlipidemic properties of *Capparis spinosa* L.: *in vivo* and *in vitro* evaluation of its nutraceutical potential. *J. Funct. Foods.* 35, 32–42. doi: 10.1016/j.jff.2017.05.001

Moubasher, H., Abd El-Ghani, M. M., Kamel, W., Mansi, M., and El-Bous, M. (2011). Taxonomic considerations among and within some Egyptian taxa of *Capparis* and related genera (Capparaceae) as revealed by RAPD fingerprinting. *Collect Bot.* 3, 29–35. doi: 10.3989/collectbot.2011.v30.003

Moufid, A., Farid, O., and and, M., Eddouks (2015). Pharmacological Properties of *Capparis spinosa* Linn. *Int. J. Diabetol. Vasc. Dis. Res.* 3, 99–104.

Mousavi, S. H., Housseini, A., Bakhtiari, E., and Rakhshandeh, H. (2016). *Capparis spinosa* reduced Doxorubicin-induced cardiotoxicity in Cardiomyoblast cells. *Avcenna J. Phytomed.* 6, 488–494.

Mouterde, P. (1968). *Nouvelle flore du Liban et de la Syrie.* Beirut: Imprimerie Catholique.

Muhaidat, R., Al-Qudah, A. M., Al-Shayeb, A., Jacob, H. J., Al-Jaber, H., Hussein, E., et al. (2013). Chemical profile and antibacterial activity of crude fractions and essential oils of *Capparis ovata* Desf. And *Capparis spinosa* L. (Capparaceae). *Int. J. Integ. Biol.* 14, 39–47.

Murzin, V. S. (1986). Diurnal Lepidoptera (Rhopalocera) of the Badkhyzskii Reserve (Turkmen, SSR). *Trudy Vsesoyuznogo Entomologisheskogo Obshchestva, Akademiya Nauk SSSR.* 6, 125–130.

Musallam, I., Duwayri, M., and Shibli, R. (2010). Micropropagation of caper (*Capparis spinosa* L.) from wild plants. *Funct Plant Sci Biotechnol.* 5, 17–21.

Nabavi, S. F., Maggi, F., Daglia, M., Habtemariam, S., Rastrelli, L., and Nabavi, S. M. (2016). Pharmacological effects of *Capparis spinosa* L. *Phytother. Res.* 30, 1733–1744. doi: 10.1002/ptr.5684

Neyisci, T. (1987). A study on the slow burning plant species suitable for controlling forest fires' (in Turkish, summary in English). *Turk. J. Agric. For.* 11, 595–604.

Nosrati, H., Feizi, H. A. M., Mazinani, M., and Haghighi, R. A. (2012). Effect of population size on genetic variation levels in *Capparis spinosa* (Capparaceae) detected by RAPDs. *EurAsia J. BioSci.* 6, 70–75. doi: 10.5053/ejobios.2012.6.0.8

Ohama, N., Sato, H., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2017). Transcriptional regulatory network of plant heat stress response. *Trends Plant Sci.* 22, 53–65. doi: 10.1016/j.tplants.2016.08.015

Oppenheimer, H. R. (1960). "Adaptation to drought; xerphytism," in *UNESCO, Plant-Water Relationships in Arid and Semi-Arid Conditions*, ed Reviews of Research (New York, NY: United Nations), 105–138.

Ortiz, R., Braun, H. J., Crossa, J., Crouch, J. H., Davenport, G., Dixon, J., et al. (2008). Wheat genetic resources enhancement by the International Maize and Wheat Improvement Center (CIMMYT). *Genet. Resour. Crop Evol.* 55, 1095–1140. doi: 10.1007/s10722-008-9372-4

Orphanides, G. M. (1975). Biology of the carob midge complex, *Asphondylia* spp. (Diptera, Cecidomyiidae) in Cyprus. *Bull. Entomol. Res*. 65, 381–390. doi: 10.1017/S0007485300006052

Orphanos, P. I. (1983). Germination of caper (*Capparis spinosa* L.) seeds. *J. Hortic. Sci.* 58, 267–270. doi: 10.1080/00221589.1983.11515119

Ozbek, O., and Kara, A. (2013). Genetic variation in natural populations of *Capparis* from Turkey, as revealed by RAPD analysis. *Plant Syst. Evol.* 299, 1911–1933. doi: 10.1007/s00606-013-0848-0

Özkahraman, I. (1997). *Caper. Forest Ministry, Various publications Series Nos 2.* Ankara: AGM publications.

Pachauri, R. K., Allen, M. R., Barros, V. R., Broome, J., Cramer, W., Christ, R., et al. (2014). "Climate change 2014: synthesis report," in *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, ed R. Pachauri and L. Meyer (Geneva: IPCC), 151.

Panico, A. M., Cardile, V., Garufia, F., Pugliaa, C., Boninaa, F., and Ronsisvalle, G. (2005). Protective effect of *Capparis spinosa* on chondrocytes. *Life Sci.* 77, 2479–2488. doi: 10.1016/j.lfs.2004.12.051

Pascual, B., San Bautista, A., Ferreros, N., Lopez-Galarza, S., and Maroto, J. V. (2003). Analysis of germination of caper seeds as influenced by the position of fruit on the mother plant, fruit maturation stage and fruit weight. *J. Hortic. Sci. Biotech* 78, 73–78.

Peri, E., Lo Bue, P., Federico, R., Ammavuta, G., Spatafora, F., and Colazza, S. (2006). *Asphondylia gennadii* (Marchal) fitofago dannoso al cappero nelle isole minori della Sicilia (Diptera: Cecidomyiidae). *Inf. Fitopatol.* 56, 26–30.

Pilone, N. (1990). Effetti dell'IBA sulla radicazione delle talee di *Capparis spinosa* in cassone riscaldato. *Inf. Agrar.* 46, 81–82.

Pittaway, A. R. (1979). The butterflies and hawk-moths of Eastern Saudi Arabia. *Proc. Br. Entomol. Nat. Hist. Soc*. 12, 90–101.

Post, G. E. (1932). *Flora of Syria, Palestine and Sinai.* Beirut: American University of Beirut.

Prasad, P. V. V., Staggenborg, S. A., and Ristic, Z. (2008). "Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants," in *Response of Crops to Limited Water: Understanding and Modeling Water Stress Effects on Plant Growth Processes*, ed L. R. Ahuja, V. R. Reddy, S. A. Saseendran, and Q. Yu (Madison, WI: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America), 301–355.

Psaras, G. K., and Sofroniou, I. (1999). Wood anatomy of *Capparis spinosa* from an ecological perspective. *IAWA J.* 20, 419–429. doi: 10.1163/22941932-90001567

Rahmani, R., Mahmoodi, M., Karimi, M., Hoseini, F., Heydari, R., Salehi, M., et al. (2013). Effect of hydroalcoholic extract of *Capparis spinosa* fruit on blood sugar and lipid profile of diabetic and normal rats. *Zahedan J. Res. Med. Sci*. 15, 34–38.

Rahnavard, R., and Razavi, N. (2016). A review on the medical effects of *Capparis spinosa* L. *Adv. Herb. Med.* 2, 44–53.

Rangarajan, A. V., and Mahadewan, N. R. (1975). Incidence of gallmidge, Asphondylia capsici Barnes on chilli in Tamil. *Indian J. Entomol.* 36, 66–67.

Rapisarda, C. (1985). Presenza in Italia di Aleurolobus niloticus Priesner & Hosny, nuovo parassita delle piante di cappero (Homoptera: Aleirodidae). *Bollettino di Zoologia agraria e Bachicoltura.* 18, 75–86.

Ray, D. K., Gerber, J. S., MacDonald, G. K., and West, P. C. (2015). Climate variation explains a third of global crop yield variability. *Nat. Commun.* 6:5989. doi: 10.1038/ncomms6989

Renfrew, J. M. (1973). *Palaeoethnobotany. The Prehistoric Food Plants of the Near East and Europe.* New York, NY: Columbia University Press.

Rhizopoulou, S. (1990). Physiological responses of *Capparis spinosa* L. to drought. *J. Plant Physiol.* 136, 341–348. doi: 10.1016/S0176-1617(11)80060-X

Rhizopoulou, S., Heberlein, K., and Kassianou, A. (1997). Field water relations of *Capparis spinosa* L. *J. Arid. Environ*. 36, 237–248. doi: 10.1006/jare.1996.0207

Rhizopoulou, S., Loannidi, E., Alexandredes, N., and Argiropoulos, A. (2006). A study of functional and structural traits of the nocturnal flowers of *Capparis spinosa* L. *J. Arid. Environ.* 66, 635–647. doi: 10.1016/j.jaridenv.2005.12.009

Rhizopoulou, S., and Psaras, G. K. (2003). Development and structure of drought-tolerant leaves of the Mediterranean shrub *Capparis spinosa* L. *Ann. Bot.* 92, 377–383. doi: 10.1093/aob/mcg149

Rivera, D., Alcaraz, F., Inocencio, C., Obón, C., and Carreño, E. (1999). "Taxonomic study of cultivated Capparis sect. Capparis in the western Mediterranean," in *Taxonomy of Cultivated Plants*, ed S. Andrew, A. C. Leslie, and C. Alexander (England: Royal Botanic Gardens), 451–455.

Rivera, D., Inocencio, C., Obon, C., and Alcaraz, F. (2003). Review of food and medicinal uses of *Capparis* L. subgenus Capparis (Capparidaceae). *Econ. Bot.* 57, 515–534. doi: 10.1663/0013-0001(2003)057[0515:ROFAMU]2.0.CO;2

Rivera, D., Inocencio, C., Obon, C., Carreno, E., Reales, A., and Alcaraz, F. (2002). Archaeobotany of Capers (Capparis) (Capparaceae). *Veg. Hist. Archaeobot*. 11, 295–314. doi: 10.1007/s003340200042

Rodriguez, R., Rey, M., Cuozzo, L., and Ancora, G. (1990). *In vitro* propagation of caper (*Capparis spinosa* L.). *In Vitro Cell. Dev. Biol*. 26, 531–536. doi: 10.1007/BF02624097

Romeo, V., Ziino, M., Giuffrida, D., Condurso, C., and Verzera, A. (2007). Flavor profile of Capers (*Capparis spinosa* L.) from the Eolian Archipelago by HS-SPME/GC-MS. *Food Chem*. 101, 1272–1278. doi: 10.1016/j.foodchem.2005.12.029

Rotondi, A., Rossi, F., Asunits, C., and Cesaraccio, C. (2003). Leaf xeromorphic adapatations of some plants of coastal Mediterranean ecosystem. *J. Meditternean Ecol.* 3, 25–35.

Saadaoui, E., Guetat, A., Tlili, N., El Gazzah, M., and Khaldi, A. (2011). Subspecific variability of Tunisian wild populations of *Capparis spinosa* L. *J. Med. Plants Res.* 5, 4339–4348.

Saadaoui, E., Khaldi, A., Khouja, M. L., and El-Gazzah, M. (2007). Etude de la variabilité morphologique du câprier (*Capparis* spp.) en Tunisie. *Revue des Régions Aride*. 2, 523–527.

Sakcali, M., Bahadir, H., and Ozturk, M. (2008). Eco-physiology of *Capparis spinosa* L. A plant suitable for combating desertification. *Pak. J. Bot.* 40, 1481–1486.

Saifi, N., Ibijbijen, J., and Echchghadda, G. (2011). Genetic diversity of Caper plant (*Capparis* sp.) from Morocco. *J. Food Agric. Environ.* 9, 299–304.

Salem, A., Zemni, H., and Ghorbel, A. (2001). Propagation of caper (*Capparis spinosa* L.) by herbaceous cuttings and *in vitro* culture. *Agric. Med.* 31, 42–48.

Selfayan, M., and Namjooyan, F. (2016). Inhibitory *Effect of Capparis spinosa* Extract on Pancreatic Alpha-Amylase Activity. *Zahedan J. Res. Med. Sci.* 18:e6450. doi: 10.17795/zjrms-6450

Sharaf, M., El-Ansari, A. M., and Saleh, N. A. M. (1997). Flavonoids of four *Cleome* and three *Capparis* species. *Biochem. Syst. Ecol.* 25, 161–166 doi: 10.1016/S0305-1978(96)00099-3

Sharaf, M., El-Ansari, A. M., and Saleh, N. A. (2000). Quercetin triglycoside from *Capparis spinosa*. *Fitoterapia.* 71, 46–49. doi: 10.1016/S0367-326X(99)00116-1

Sheikh, K. H. (1976). "Variations in leaf hydration and stomatal openings of some maquis in response to changes in some environmental factors," in *Proceedings of the Third Mediterranean Plant Physiology Meeting,* ed Y. Vardar, K. H. Sheikh, and M. Ozturk (Izmir: Ege University Press), 24–36.

Sher, H., Al-Mutayri, K., and Mansoor, M. (2012). Study on the ethnopharmaceutical values and traditional uses of *Capparis spinosa* L. *Afr. J. Pharm. Pharmamcol.* 6, 1255–1259.

Sher, H., and Alyemeni, M. (2010). Ethnobotanical and pharmaceutical evaluation of *Capparis spinosa* L, validity of local folk and Unani system of medicine. *J. Med. Plants Res.* 4, 1751–1756.

Silvestre, G. A., Silvio, F., Mirko, S., Ignazio, F., Giuseppe, G., and Francesco, C. (2014). Hybridization in *Capparis spinosa* L.: molecular and morphological evidence from a Mediterranean island complex. *Flora* 209, 733–741. doi: 10.1016/j.flora.2014.09.002

Singh, R. P., Bahar, N., and Chand, P. (1992). Autecology of *Capparis spinosa* Linn. in cold desert of Spiti Valley in Himachal Pradesh. *Ann. Arid. Zone*. 31, 291–293.

Solowey, E. (2010) "*Arboreal pastures*" *in Growing Bread on Trees: The Case for Perennial Agriculture*. Miami, FL: Acco, Israel/Biblio Books International.

Sozzi, O. G. (2001). Caper bush: botany and horticulture. *Hortic. Rev.* 27, 125–188. doi: 10.1002/9780470650813.ch4

Sozzi, O. G., and Chiesa, A. (1995). Improvement of caper (*Capparis spinosa* L.) seed germination by breaking seed coat-induced dormancy. *Sci. Hortic.* 62, 255–261. doi: 10.1016/0304-4238(95)00779-S

Sozzi, O. G., and Vicente, A. R. (2006). "Capers and caperberries," in *Handbook of Herbs and Spices*, ed K. V. Peter (Boca Raton, FL: Woodhead Publishing Limited; CRC Press), 230–256.

Stefanou, M., and Manetas, Y. (1997). The effect of season, exposure, enhanced UV-B radiation, and water stress on leaf epicuticular and internal UV-B absorbing capacity of *Cistus creticus*: a Mediterranean field study. *J. Exp. Bot.* 48, 1977–1985. doi: 10.1093/jxb/48.11.1977

Thiry, A. A., Dulanto, P. N. C., Reynolds, M. P., and Davies, W. J. (2016). How can we improve crop genotypes to increase stress resilience and productivity in a future climate? A new crop screening method based on productivity and resistance to abiotic stress. *J. Exp. Bot*. 67, 5593–5603. doi: 10.1093/jxb/erw330

Tlili, N., El-Fallah, W., Saadadoui, E., Khaldi, A. H., Triki, S., and Nasri, N. (2011a). The caper (*Capparis* L.): ethnopharmacology, phytochemical and pharmacological properties. *Fitoterapia* 82, 93–101. doi: 10.1016/j.fitote.2010.09.006

Tlili, N., El Guizani, T., Nasri, N., Khaldi, A., and Triki, S. (2011b). Protein, lipid, aliphatic and triterpenic alcohol content of Caper seeds "*Capparis spinosa*". *J. Am. Oil Chem. Soc*. 88, 265–270. doi: 10.1007/s11746-010-1662-2

Tlili, N., Feriani, A., Saadoui, E., Nasri, N., and Khaldi, A. (2017). *Capparis spinosa* leaves extract: source of bioantioxidants with nephroprotective and hepatoprotective effects. *Biomed. Pharmacother*. 87, 171–179. doi: 10.1016/j.biopha.2016.12.052

Tlili, N., Khaldi, A., Triki, S., and Munné-Bosh, S. (2010). Phenolic compounds and vitamin antioxidants of Caper (*Capparis spinosa*). *Plant Foods Hum. Nutr.* 65, 260–265. doi: 10.1007/s11130-010-0180-6

Tomassoli, L., Zaccaria, A., and Barba, M. (2005). *Capparis spinosa*, a new host of *cucumber mosaic virus* in Italy. *Plant Pathol. J.* 54:263. doi: 10.1111/j.1365-3059.2005.01125.x

Trombetta, D., Occhiuto, F., Perri, D., Puglia, C., Santagati, N. A., Pasquale, A. D., et al. (2005). Antiallergic and antihistaminic effect of two extracts of *Capparis spinosa* L. flowering buds. *Phytother. Res*. 19, 29–33. doi: 10.1002/ptr.1591

UNEP/ROWA (United Nations Environment Programme, Regional Office for West Asia) (2015). *Climate Change in the Arab Region. Regional Coordination Mechanism. Issues Brief for the Arab Sustainable Development Report.* Manama.

Vahid, H., Rakhshandeh, H., and Ghorbani, A. (2017). Antidiabetic properties of *Capparis spinosa* L. and its components. *Biomed. Pharmacother*. 92, 293–302. doi: 10.1016/j.biopha.2017.05.082

Van, Z. W., and Bakker-Heeres, J. A. H. (1988). Archaeobotanical studies in the Levant. 4. Bronze Age sites on the North Syrian Euprates. *Palaeohistoria* 27, 247–316.

Vardar, Y., and Ahmed, M. (1972). Relative transpiration of the old and young leaves of some macchina elements. *Phyton* 14, 251–262.

Velikova, V., Pinelli, P., Pasqualini, S., Reale, L., Ferranti, F., and Loreto, F. (2005). Isoprene decreases the concentration of nitric oxide in leaves exposed to elevated ozone. *New Phytol.* 166, 419–426. doi: 10.1111/j.1469-8137.2005.01409.x

Vollenweider, P., and Günthardt-Goerg, M. S. (2005). Diagnosis of abiotic and biotic stress factors using the visible symptoms in foliage. *Environ. Pollut.* 137, 455–465. doi: 10.1016/j.envpol.2005.01.032

Wahid, A. (2007). Physiological implications of metabolites biosynthesis for net assimilation and heat- stress tolerance of sugarcane (*Saccaharum officinarum)* sprouts. *J. Plant Res.* 120, 219–228. doi: 10.1007/s10265-006-0040-5

Wang, Q., Zhang, M. L., and Yin, L. K. (2016). Phylogeographic structure of a tethyan relict *Capparis spinosa* (Capparaceae) traces pleistocene geologic and climatic changes in the western Himalayas, Tianshan mountains, and adjacent desert regions. *Biomed. Res. Int.* 2016:13. doi: 10.1155/2016/5792708

Wealth of India (1992). *A Dictionary of Indian Raw Material and Industrial Products*. New Delhi: CSIR.

Willis, J. C. (1988). *A Dictionary of the Flowering Plants and Ferns.* Cambridge: Cambridge Cambrigde University Press.

Xu, Z., Jiang, Y., and Zhou, G. (2015). Response and adaptation of photosynthesis, *respiration*, and antioxidant systems to elevated CO_2 with environmental stress in plants. *Front. Plant Sci*. 6:701. doi: 10.3389/fpls.2015.00701

Yamori, W., Hikosaka, K., and Way, D. A. (2014). Temperature response of photosynthesis in C3, C4, and CAM plants: temperature acclimation and temperature adaptation. *Photosynth. Res.* 119, 101–117. doi: 10.1007/s11120-013-9874-6

Yang, T., Wang, C., Chou, G. X., Wu, T., Cheng, X. M., and Wang, Z. T. (2010b). New alkaloids from *Capparis spinosa*: structure and X-ray crystallographic analysis. *Food Chem.* 123, 705–710. doi: 10.1016/j.foodchem.2010.05.039

Yang, T., Wang, C., Liu, H., Chou, G., Cheng, X., and Wang, Z. (2010a). A new antioxidant compound from *Capparis spinosa*. *Pharm. Biol.* 48, 589–594. doi: 10.3109/13880200903214231

Yu, L., Yang, J., Wang, X., Jiang, B., Sun, Y., and Ji, Y. (2017). Antioxidant and antitumor activities of *Capparis spinosa* L. and the related mechanisms. *Oncol. Rep*. 37, 357–367. doi: 10.3892/or.2016.5249

Zhang, S., Hu, D. B., He, J. B., Guan, K. Y., and Zhu, H. J. (2014). A novel tetrahydroquinoline acid and a new racemic benzofuranone from *Capparis spinosa* L., a case study of absolute configuration determination using quantum methods. *Tetrahedron* 70, 869–873. doi: 10.1016/j.tet.2013.12.024

Zhou, H., Jian, R., Kang, J., Huang, X., Li, Y., Zhuang, C., et al. (2010). Anti-inflammatory effects of Caper (*Capparis spinosa* L.) fruit aqueous extract and the isolation of main phytochemicals. *J. Agric. Food Chem.* 58, 12717–12721. doi: 10.1021/jf1034114

Zhou, X., and Liu, Y. (2015). Hybridization by grafting: a new perspective? *HortScience* 50, 520–521.

Ziroyan, A. N. (1980). Seed productivity and renewal of some semidesert plant species on the large southern slope of Mount Aragats, Armenian SSR, USSR' (in Russian). *Biol. Zh. Arm.* 33, 91–94.

Zohary, M. (1960). The species of *Capparis* in the Mediterranean and the Near Eastern countries. *Bull. Res. Counc. Isr*. 8D, 49–64.

Zuo, W., Ma, M., Ma, Z., Gao, R., Guo, Y., Jiang, W., et al. (2012). Study of photosynthetic physiological characteristics of desert plant *Capparis spinosa* L. *J. Shihezi Univ*. 3:006.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Environmental Science

ORIGINAL RESEARCH
published: 18 September 2018
doi: 10.3389/fenvs.2018.00102

Artificially Induced Floods to Manage Forest Habitats Under Climate Change

Berit Arheimer*, Niclas Hjerdt and Göran Lindström

Swedish Meteorological and Hydrological Institute, Norrköping, Sweden

Global change is affecting agroforestry and its inherent ecosystems in Sweden. Here we examine the benefits of ecologically adjusted dam regulations to conserve biodiversity under climate change in floodplain habitats, including meadows and riparian mixed forests. The natural flood regime in snow-dominated regions has changed significantly during the last decades, in line with the projections for climate change. The ecosystems of temporary flooded forests show high biodiversity but are dependent on river high flows with long duration. These events are rare in the new climate scenario, but on the other hand, snow-fed rivers are also affected by hydropower dams and regulations. In this study we explored the potential of using reservoir regulation to artificially induce flood events; water management would then be a method to conserve biodiversity in forest habitats and adapt management to climate change. We made detailed calculations in lower Dalälven River, central Sweden, using observed time-series of river flow and dynamic scenario modeling for highly valuable Natura 2000 habitats. Here we show that long-term flooding is less frequent since extensive hydropower was introduced during the 1920s, and moreover, since the 1990's the spring floods are low due to low snow storage and short winter seasons. Sustainable management of 50% of the riparian forest requires flooding by 25 continuous days of 800 m^3 s^{-1}. We found that artificial floods using new ecological regulation regime of upstream hydropower reservoirs would help, but not be enough, to achieve this goal. The new regulation routines would correspond to a loss of 50-200 GWh in hydropower production for each artificial flood. Sustainable ecosystems in the study site do not request flooding every year, but some every fifth year. For practical implementation, the County Board is currently driving the process locally and we discuss the relevant social features, such as legal and funding aspects, of this adaptive management of water and forests. A smaller part of the forest could probably be rescued and costs could potentially be lowered by using only the most snow rich years and seasonal forecasting of river flow for optimal timing of water release from dams to induce flooding.

Keywords: environmental flow, river regulation, climate change, climate adaptation, biodiversity, water management, floodplains

Edited by:
José Manuel Mirás-Avalos,
Universidade de Santiago de Compostela, Spain

Reviewed by:
Fei WANG,
Institute of Soil and Water Conservation (CAS), China
Aitor García Tomillo,
University of A Coruña, Spain

****Correspondence:***
Berit Arheimer
Berit.Arheimer@smhi.se

Specialty section:
This article was submitted to Agroecology and Ecosystem Services, a section of the journal Frontiers in Environmental Science

Received: *03 March 2018*
Accepted: *23 August 2018*
Published: *18 September 2018*

Citation:
Arheimer B, Hjerdt N and Lindström G (2018) Artificially Induced Floods to Manage Forest Habitats Under Climate Change. Front. Environ. Sci. 6:102. doi: 10.3389/fenvs.2018.00102

INTRODUCTION

Currently, productive forests account for 57% of the Swedish land cover and they have been constantly expanding throughout the 20th century (KSLA, 2015; Nilsson et al., 2016). During the last century, forests in Sweden have seen important changes in forest structure and composition (Antonson and Jansson, 2011). Agroforestry is responsible for most changes in the diversity of coniferous and deciduous species (Laudon et al., 2011; Elmhagen et al., 2015), yet, climate change and water management may also have a severe impact on biodiversity in these forests. The highest biodiversity in Sweden is found in ecotopes of temporarily flooded riparian mixed forests, which have been recognized as highly valuable Natura 2000 habitats (Hedström-Ringvall et al., 2017a). The high biodiversity is dependent on river high flows with long duration, but such occasions have become rare during the last decades. As a consequence, there is a great concern about the possible disappearance of these valuable ecosystems.

The flood regime in snow-dominated regions has changed significantly during the last decades, which is in line with the projections for climate change impact but also an effect of extensive flow regulations (Arheimer et al., 2017). During the cold part of the year in Sweden, water is stored as snow and ice, which fully or partly melt during the spring. The natural flow regime is therefore characterized by low flow during the winter followed by a high spring peak flood event. The ecosystems have evolved over time to benefit from these flow dynamics with high biodiversity in the floodplains as an outcome. Several studies of climate-change impacts on rivers show that the annual peak flood event may be less distinct and even disappear in some snow dominated areas (Molini et al., 2011; Godsey et al., 2013) as global warming will reduce the snow fall (Krasting et al., 2013) and/or snow storage period by the end of this century (Barnett et al., 2005). More precipitation falling as rain in snow-dominated regions and shorter freezing periods will thus give less difference in river flow between seasons and less flooding of floodplains during spring.

Arheimer et al. (2017) concluded that at the large scale and for floodplains in snow-dominated regions, hydropower production can have the same effect as climate change on the flow regime. During spring, the river water is stored in dams and reservoirs often to be released throughout the year whenever electricity is needed most. Thus, the high flow of the snow-melt season is damped and the river flow redistributed to other times of the year (e.g., Arheimer and Lindström, 2014). The negative effects on ecosystems that follow from river regulation are well known (e.g., Andersson et al., 2000; Bunn and Arthington, 2002; Leira and Cantonati, 2008) and in addition, ecosystems in regulated rivers are considered particularly vulnerable to climate change (e.g., Nilsson et al., 2005; Palmer et al., 2008). However, ecosystems affected by river regulations have also been suggested as more favorable for adaptation measures as flow regimes can be manipulated (e.g., Lytle and Poff, 2004; Rheinheimer and Viers, 2015). Artificially induced floods by changed regulation regimes at hydropower dams have been suggested as a possible climate adaptation measure to conserve biodiversity under climate change (Arheimer et al., 2017). Nevertheless, the potential of this management protocol still remains unknown and detailed site-specific investigations are needed in each case to justify any implementation, as large economic costs are involved from loss in energy production.

We here describe such a case study of central Sweden, where detailed calculations were made to: (i) analyse the reasons behind the reduced flooding that threatens a rare forest habitat, and (ii) explore the potential of using changed regulation strategy at upstream hydropower dams to induce river flooding for sustainable management. We found that reduction of snow storage was the main reason behind the loss of peak-flows during recent decades, and that changed regulation of hydropower dams could not save 50% of the habitats of the threatened temporarily flooded forest. We show that the regulations could help in climate adaptation, but there may be high costs for energy loss and melt-water must still be available in sufficient amounts from snow storage. Overall, the study highlights the importance of revising management protocols under non-stationary conditions due to global warming.

MATERIALS AND METHODS

Study Site

The floodplain forests of lower Dalälven River in central Sweden, receive water from a 29,000 km^2 watershed starting in the mountains of Norway to the west, from which the river flows to the east and ends in the Baltic Sea (**Figure 1**). The regulation of Dalälven River started during the 1920's to produce electricity from hydropower reservoirs. Dams were constructed to store the water from snowmelt during spring, to be released for hydropower production throughout the year, especially during the long and dark winter when most electricity is needed in this region. Often natural lakes are regulated but in some cases new reservoirs were constructed. Altogether, the watershed encompasses about 125 dams at present, with a total regulated volume of 2,739 Mm^3 and a degree of regulation of 23.5% (Arheimer et al., 2017). The total annual production is approximately 5 TWh year^{-1}. The most prominent dams are Gråda and Trängslet. Gråda is regulating the lake Siljan and was constructed during the 1920's with a head of 12 meters and volume of 660 Mm^3 (Hedström-Ringvall et al., 2017b). Trängslet is from 1950's with a head of 142 meters and a volume of 880 Mm^3. Both are owned by the company Fortum and the regulation schemes also affect many down-stream dams.

Several climate change impact studies have encompassed river flow in Dalälven River (e.g., Andréasson et al., 2004; Arheimer et al., 2017) and can also be found at the websites www.smhi.se or http://swicca.eu/. The climate change projections show an increase in mean annual air temperature by +3 to +6°C at the end of the Twenty-First century, with a higher increase in winter (+4 to +7°C) compared to summer temperatures (+2 to +4°C). At the same time, annual precipitation is expected to increase by ±0 to +30%, with a higher increase in winter (+20 to +40%) compared to summer precipitation (±0 to +20%). The growing season is expected to increase by 40 to 80 days at the end of the Twenty-First century.

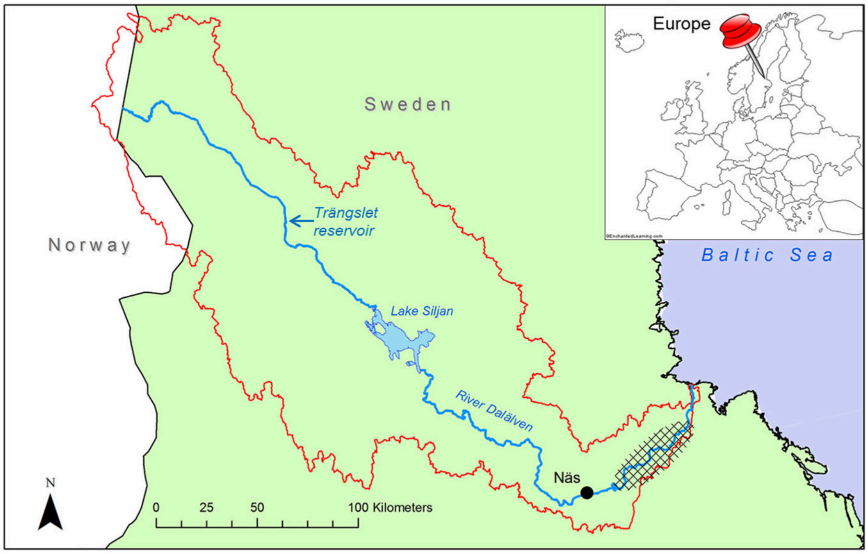

FIGURE 1 | Map showing the catchment borders (red line) of Dalälven River (blue line) in Northern Europe, and the location of the hydropower plant "Näs" (black dot) upstream the regularly flooded residua forest of high biodiversity (checkered).

Environmental Flows for Forest Flooding

The natural river regime with annual floods due to snowmelt during the spring has created a very unique zonation of ecosystems at various altitudes of the floodplains, depending on frequency and duration in flooding. The region is recognized as having the highest biodiversity in Sweden, with several valuable Natura 2000 habitats identified along the river floodplains. This is a unique service provided by the river from its original flow dynamics. The most vulnerable ecosystem is the regularly flooded riparian mixed forests, which requests flood duration of 25 continuous days to initiate the ecological processes and serve as habitat for the specific species living and growing there (Hedström-Ringvall et al., 2017b).

Differentiation of floodplain habitats was based on different elevation zones, using a topographic GIS analysis (Zinke, 2013). Each habitat needs specific flooding to sustain the diversity of species and different elevation zones will be flooded at different magnitudes of river flow. As most of floodplain habitats are located along wide, lake-like, sections of the river, the river flows required to flood these habitats were estimated from an analysis of habitat elevation zones and stage-discharge rating curves of the lake outlets (**Figure 2**). The topographical GIS analysis of the floodplains together with hydraulic modeling results show that river flow above 800 m^3s^{-1} will flood a significant part of the riparian mixed forest (the mean elevation minus one standard deviation, i.e., 56.4 m, see Table 5, 6 by Zinke, 2013), while 1,250 m^3s^{-1} is needed for all forest representing these unique ecotopes to be flooded (Hedström-Ringvall et al., 2017a). In this study, the value 800 m^3s^{-1} was thus used as environmental flow for forest flooding to conserve the biodiversity in lower Dalälven River.

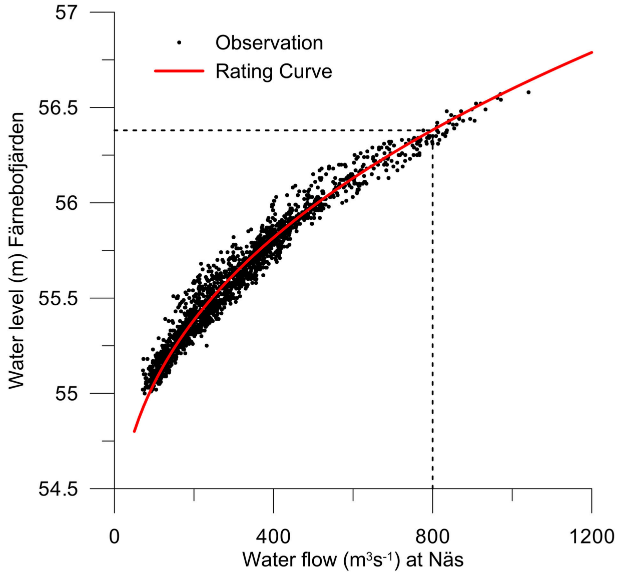

FIGURE 2 | Analysis of floodplain habitat elevation zones in relation to the observed and modeled stage-discharge rating curve for Färnebofjärden in Lower Dalälven. In order to flood riparian mixed forest habitats (Natura 2000 habitat code 91F0) a river discharge exceeding 800 m^3s^{-1} is required.

Data and Methods

Observed time-series of daily river flow were collected from the discharge stations at Långhag/Fäggeby (since 1851) and at Näs (since 1961). The stations are situated not far from each other in the main river channel close to the downstream floodplains. The observed data was used to analyse the long-term changes in river flow, which could be due to either climate variability/change or flow regulation in hydropower dams. Observed time-series from the hydropower stations Trängslet and Gråda (Lake Siljan) were used as a base-line when exploring effects from changed regulation routines to induce flooding for specific years (see below).

Dynamic modeling was performed using the numerical Hydrological Predictions for the Environment (HYPE) model (Lindström et al., 2010) as set up for Sweden in S-HYPE (Strömqvist et al., 2012). The model simulates flow generating processes from meteorological input data, by taking into account for instance snow melt, evapotranspiration, soil moisture, groundwater fluctuations, routing in lakes and streams. It also includes routines for simulating regulation in hydropower reservoirs (Arheimer and Lindström, 2014; Arheimer et al., 2017). In the national model setup, the Dalälven River basin is divided into 2,823 coupled watersheds along the river network.

The S-HYPE model was used to simulate the changes in snow storage between 1961 and 2015, to evaluate the impact from climate on observed changes in river regime. The S-HYPE model was also used to reconstruct the natural river flow, as it would have been without the influence of hydropower regulations, using the method described by Arheimer and Lindström (2014) and Arheimer et al. (2017). Return periods for daily environmental flows, were analyzed by dividing time series data into 30-year periods and using the statistical method described by Bergstrand et al. (2014). Time series from two sources were used: Observed daily discharge from Långhag/Fäggeby for the period 1851–1920 (before the development of hydropower), and reconstructed natural daily discharge for Näs for the period 1961–2015 (after the development of hydropower), using the hydrological model S-HYPE.

To investigate future climate impact on river flow, the S-HYPE model was used for climate change projections. It was then fed with time-series from the Coupled Model Intercomparison Project Phase 5 (CMIP5) from the Intergovernmental Panel on Climate Change (IPCC), using projections for two different assumptions on societal development and emission scenarios (Representative Concentration Pathways (RCPs) 4.5 and 8.5, respectively). Data was extracted from the Regional Climate model RCA (Samuelsson et al., 2011) version 4, using an ensemble of nine General Circulation Models (GCM): CanESM2, CNRM-CM5, GFDL-ESM2M, EC-EARTH, IPSL-CM5A-MR, MIROC5, MPI-EMS-LR, NorESM1-M, HadGEM2-ES. The RCA downscaled the GCM data from 1,000 km to 50 km, as part of the Coordinated Regional Downscaling Experiment (CORDEX) initiative (http://www.cordex.org/). Thereafter statistical bias-correction was made using the distributed based scaling (DBS) method (Yang et al., 2010) to a Swedish 4 km meteorological grid based on observations (Johansson, 2002). To estimate future climate change impact on Dalälven River, the river flow at the end of the century (2068–2098) was compared with a reference period (1981–2010) for each ensemble member.

To explore the effects from inducing floods by changed regulation strategies, four alternative scenarios were constructed for the spring flood of recently wet years (1999, 2006, 2010, 2015):

- Business as usual, using daily corrected observations from monitoring stations by the Swedish Meteorological and Hydrological Institute (SMHI).
- Scenario 1, reduced regulation from both Trängslet and Siljan hydropower dams.
- Scenario 2, reduced regulation only from Lake Siljan hydropower dam (Gråda).
- Natural flow calculated with the S-HYPE model as described by Arheimer and Lindström (2014) and Arheimer et al. (2017).

The calculations at both Trängslet and Siljan hydropower dams were made by the hydropower companies (Hedström-Ringvall et al., 2017a), using site-specific and detailed local information on regulation capacities, head, outlet conditions, and legal agreements on volumes, water levels, spill, and flow (Hedström-Ringvall et al., 2017b). They used their operational set-up of numerical models for production, based on the open access tool Hec ResSim from US Army Corps of the Engineers (http://www.hec.usace.army.mil/software/hec-ressim/) as applied for the specific reservoirs. Additional lumped flow routing methods were used, as suggested by Chow et al. (1988). For scenario 1 and 2, the flows to downstream reservoirs were adjusted by applying the estimated change in flow from calculated regulations at Trängslet or Siljan to the observed flows at each site.

RESULTS

Observed Changes in River Flow

Long-term flooding is less frequent since extensive hydropower was introduced during the period 1920–1960 and the spring peak is less pronounced. Time-series of >50 years of observations both before and after building hydropower dams, show that the average in high water inflow causing flooding of the vulnerable forest habitat has dropped from 900 to 500 m^3s^{-1} due to regulation (**Figure 3**). The long-term average in flow is thus far from reaching the 800 m^3s^{-1} threshold for the modern time period, although specific years still exceed this threshold. Since the 1990's the annual spring-floods have been low due to low snow storage and short winter seasons. The spring peak also starts about 1 month earlier. These are results of a warming climate, which has already affected the flow regime in this region considerably. From the observed time-series, however, it is not possible to judge whether the observed change in flow regime is due to river regulations or climate change. However, our analysis of reconstructed natural flow using a hydrological model enables us to evaluate the potential effects of climate change separately. A comparison of measured flow before regulation (1852–1919) to reconstructed natural flow (1961–2015) clearly shows that the magnitude and duration of the spring-flood has decreased over the last decades.

Observed Changes in Snow Storage

Annual precipitation over the watershed upstream Näs hydropower station varies from 470 to 860 mm $year^{-1}$, but there is a slight but significant increase in average annual precipitation over the last 55 years (**Figure 4A**). Our analysis shows that while annual precipitation increases over time, the fraction of precipitation falling as snow decreases and annual maximum snow storage decreases (**Figures 4A,B**). For unregulated areas, the reduced peak-flow during spring is thus a result from reduced snow-melt and not from reduced precipitation.

The annual maximum snow storage is a relatively good indicator of the spring flood volume, and therefore also flood duration (**Figure 4C**). Years with large maximum snow storage typically yield spring floods of long duration ($R^2 = 0.56$, **Figure 4C**). On average, a maximum snow storage of about 160 mm is required to produce a spring flood that exceeds 25 days in duration, which is required to conserve 50% of the temporarily flooded riparian mixed forest. We found nine such years for the last 55 years, which could then be about enough for generating a designed flow peak of sufficient duration every fifth year.

Changes in Return Periods of Environmental Flow

There is a temporal variability in calculated return periods but more so for floods with long duration (**Figure 5**). Daily discharge above 800 m^3s^{-1} occurs on average every or every other year throughout the entire time period. Daily discharge above 800 m^3s^{-1} for at least 25 consecutive days in April-June occurred every 2–3 years in the period 1851–1920, but has become rarer after 1990. In the observed discharge data from Näs, spring-floods with such high magnitude and duration have not occurred since the mid 1980s. However, in the reconstructed natural discharge for the last 30-year period investigated (1986–2015),

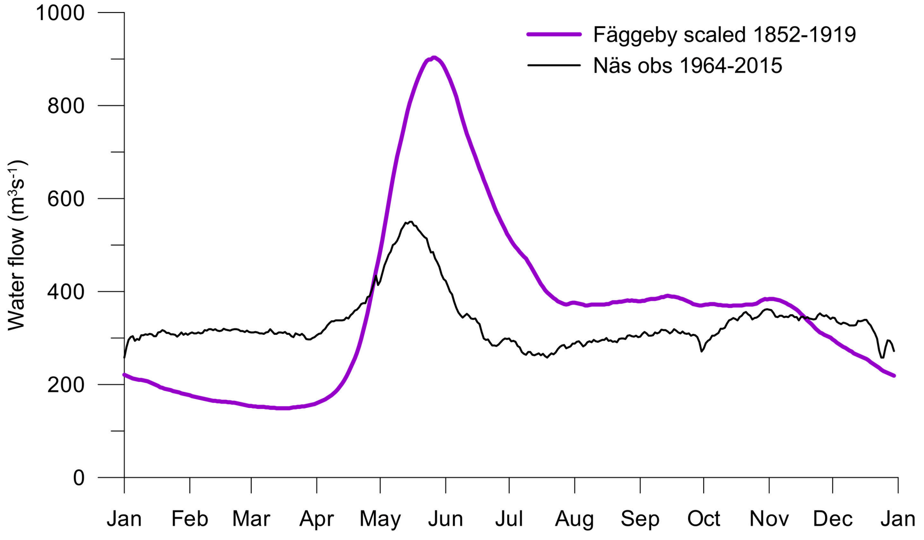

FIGURE 3 | Average water discharge at Näs, using scaled observation from the nearby river gauge at Fäggeby for the period before constructing hydropower dams (purple) and observations at Näs after regulations (black).

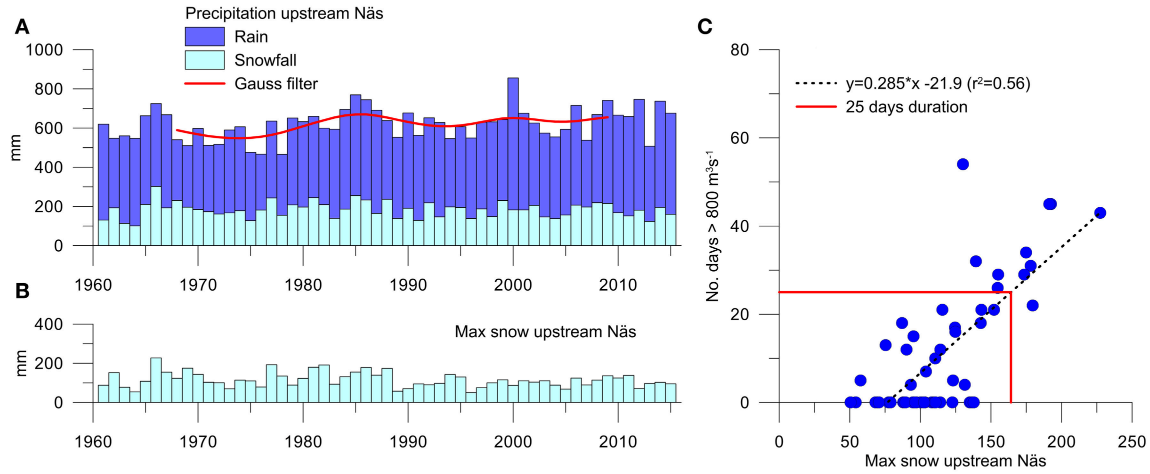

FIGURE 4 | Annual precipitation in Dalälven watershed upstream of Näs with fractions falling as rain and snow (**A, upper left**), annual maximum snow storage upstream of Näs (**B, lower left**), correlation between flood duration (continuous number of days of unregulated flow exceeding 800 m^3s^{-1} during the period between April and June) and annual maximum snow storage upstream Näs (**C, right**).

spring floods of long duration occurred every 6 years on average (**Figure 5**). This indicates again a change in climate and that future climate change may further reduce the potential for the targeted environmental flows.

Projected Future Climate Change Impact

Projections for Dalälven River suggest that climate change upstream the temporary flooded riparian mixed forest has less impact on the seasonal distribution of flow than current hydropower regulation (**Figure 6**). On average for the 30-year period studied, the natural spring flow has been reduced by 32% due to dam constructions (green vs. black line in **Figure 6**), while climate change is projected to reduce a natural spring peak by 13% (blue vs. red line in **Figure 6**). The results from this analysis show that changes in hydropower regulation control the flow more than climate. This implies that changes in management protocols could be an efficient method to reconstruct the natural flows by opening the dam gates during the flow peak in spring. Although, it is not clear if the effect would be enough.

Scenarios of Artificially Induced Floods

Although the changed strategies for regulating river flow helped to flood the riparian mixed forest of the floodplain, it would not have been possible to obtain 25 consecutive days with the environmental flow of > 800 $m^3\ s^{-1}$ at Näs during the wet years studied using any of the alternative scenarios (**Table 1**). Even for natural flow, the threshold of 25 days was only achieved during one of the years with extreme high flows (i.e., 2010). The difference in river flow between the two alternatives was not very large; however, the loss in energy production varied considerably (with a loss of some 50–200 GWh, respectively) with alternative No. 2 being much more cost effective (Hedström-Ringvall et al., 2017a). Thus, from only considering these two alternatives and losses in production, only changing the regulation of Lake Siljan came out as the best choice. However, additional challenges with changing this regulation routine include new legal agreements and security design, as well as extremely good monthly river flow forecasts.

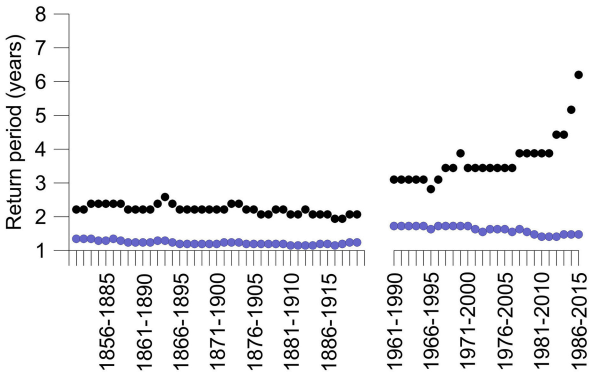

FIGURE 5 | Temporal variability in the return period of daily discharge above 800 m^3s^{-1} (blue) and daily discharge above 800 m^3s^{-1} for at least 25 consecutive days in April-June (black).

DISCUSSION

This detailed site-specific investigation for adapting the vulnerable riparian mixed forest at the floodplains of Dalälven River to climate change conditions, shows that induced floods by changed hydropower regulation will not help saving 50% of the habitats. The environmental goals must thus be revised to be realistic under climate change, as the snow storage will most likely be further reduced in the future. Sustainable management of the study site does not request flooding every year, but some every fifth year (Hedström-Ringvall et al., 2017a). The most favorable years could be chosen from snow measurements during winter and seasonal forecasts of the flow peak during spring. Monthly seasonal forecasts of spring flow show skills in the region (Arnal et al., 2017; Foster et al., 2017), although low, and the efficiency of the artificially induced floods will increase with help of natural high flow also from unregulated areas contributing to flooding of the floodplains.

The County Board is currently driving the process locally and will proceed by establishing a working group for the next 5 years to further analyze effects and potential of changed regulations. The environmental flows will be reconsidered regarding area to be flooded, to also investigate the possibilities for sustainable management of smaller areas, which requests lower river flow. Besides from the flooded riparian mixed forest there are also flooded meadows of concern that request lower flow volumes than the forest to become sustainable under climate change.

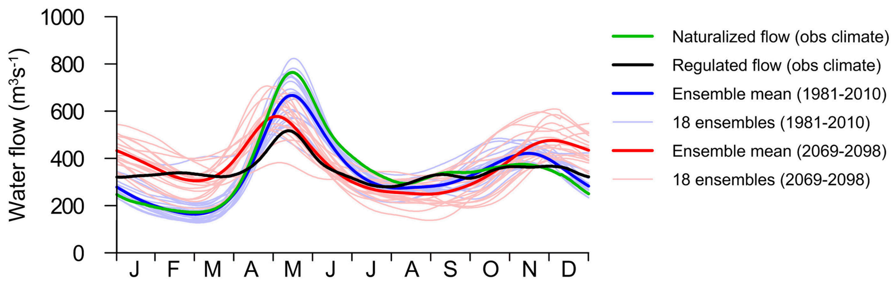

FIGURE 6 | Impact on river flow at Näs by hydropower regulation and climate change, respectively. Regulated flow is observed river flow, while naturalized flow is modeled using the observed climate (green) and the climate model data of current climate (blue) and by the end of the century (red).

TABLE 1 | Number of days and production losses for different scenarios of artificially induced floods (modified from Hedström-Ringvall et al., 2017a).

Scenario:	No. days with river flow > 800 m^3 s^{-1} at Näs				Loss of energy production (GWh)			
	1999	2006	2010	2015	1999	2006	2010	2015
B.a.U.[1]	0	0	7	4	NA	NA	NA	NA
Alt. No 1	14	6	15	12	190	174	200	215
Alt. No 2	14	5	11	7	54	47	35	54
Natural[2]	19	16	26	15	NA	NA	NA	NA

NA, Not Applicable.

[1] *Business as Usual, using present regulation strategy.*

[2] *Natural flow, without any regulation.*

Both environmental goals will be negotiated and optimized against loss in energy production in new scenarios by the working group, to estimate the most cost effective climate adaptation for floodplains in Dalälven River. In addition, a committee for adaptive management will be established to elaborate operational decision-making of artificial flooding, taking fictive decisions from various sources of support material during spring each of the 5 years.

Finally, it should be mentioned that the hydropower companies and the engineering consultants that have been involved in the calculations for each hydropower dam are more reluctant to changes in regulation strategies. The calculations for each dam were based on statistics and observations, but in reality it would be very difficult to forecast exactly when the flow peaks will reach the riparian mixed forest. There is a high risk that the gates are opened too early or too late, which significantly would affect the result. It is thus difficult to match the flood peak from artificial flooding with the natural flooding from unregulated areas, while the joint effect is needed. The dam operators also see the difficulties in spring-flood forecasting and claim that the methods available are still too poor to be used for decision-making. They also see security risks, as when the discharge from Lake Siljan once has started it will be difficult to stop, due to the naturally inherent slowness of the system, and intense rains may challenge the upper limit of the reservoir. The new regulation strategies must thus also be analyzed from a security perspective as the dam was never designed for this purpose and the legal agreements on volume fluctuations must be further validated.

Apart from the concerns about ecology, actual costs and security mentioned here, there are also other policy concerns with changing regulation strategy from hydropower dams. Hydropower is referred to as a clean and renewable energy source, which is favored over fossil fuels. Reservoir storage is often used to balance out fluctuations in other renewable power sources, such as wind and solar, which may become more important in future energy production. Hence, climate mitigation may request more hydropower in the future and more flexible regulation schemes also taking this aspect in concern. Water governance always require collaborations among multiple actors to ensure sustainability in various sectors (Falkenmark and Molden, 2008; Palmer et al., 2009; Grafton et al., 2013). Also other stakeholders representing domestic, industrial, agricultural or recreation interests may have an opinion on regulation strategies, so probably a wider audience must be addressed and consulted during the upcoming 5 years before actual implementation.

CONCLUSIONS

Our analysis show that annual maximum snow storage in Dalälven River decreases despite an overall slight increase in annual precipitation during the last 55 years, and that these changes can be attributed to climate change. During the same period, hydropower regulations have reduced the flow peaks from snow melting, which naturally should overflow the floodplains. Both changes will affect forest habitats.

Searching for sustainable agroforestry requires an analysis where hydrologists and ecologists work in close collaboration. In lower Dalälven River, riparian biodiversity relies on occasional spring floods with relatively long duration to “reset” habitats. Artificially induced flooding is one possible adaptation measure, although it implies significant costs in lost energy production and changes in both regulation strategies and river basin management plans.

Managing floodplain ecosystems under climate change is facilitated by hydrological modeling tools. In this study we demonstrate that reference conditions are not stationary under climate change, which prevents the use of historic measurements to define reference conditions and targets of river basin management. Rather, reference conditions must be dynamically modeled to be comparable to the present-day situation and for separating the anthropogenic pressures from natural variability.

AUTHOR CONTRIBUTIONS

BA outlined the manuscript, analyzed the results, and wrote the text. NH collaborated with local stakeholders and made statistical analysis. GL contributed with hydrological modeling, plots, and graphs.

ACKNOWLEDGMENTS

We would like to thank all participants in the collaborative project Sustainable hydropower in Dalälven River initiated by the Swedish Authority of Marine and Water Management; especially Per-Erik Sandberg (County board of Dalarna), Joel Berglund (County board of Uppsala), Anna Hedström-Ringvall (Regulation company of Daläven River), Claes Kjörk and Kent Pettersson (Fortum), Magnus Engström and Dag Wisaeus (ÅF/Vattenfall).

REFERENCES

Andersson, E., Nilsson, C., and Johansson, M. E. (2000). Effects of river fragmentation on plant dispersal and riparian flora. *Regulated Rivers Res Manage.* 16, 83–89. doi: 10.1002/(SICI)1099-1646(200001/02)16:13.3.CO;2-K

Andréasson, J., Bergström, S., Carlsson, B., Graham, L. P., and Lindström, G. (2004). Hydrological change–climate change impact simulations for Sweden. *AMBIO* 33, 228–234. doi: 10.1579/0044-7447-33.4.228

Antonson, H., and Jansson, U. (eds) (2011). *Agriculture and Forestry in Sweden Since 1900: Geographical and Historical Studies.* Stockholm: The Royal Swedish Academy of Agriculture and Forestry.

Arheimer, B., Donnelly, C., and Lindström, G. (2017). Regulation of snow-fed rivers affects flow regimes more than climate change. *Nat. Commun.* 8:62. doi: 10.1038/s41467-017-00092-8

Arheimer, B., and Lindström, G. (2014). "Electricity vs ecosystems–understanding and predicting hydropower impact on swedish river flow. evolving water resources systems: understanding, predicting and managing water–society interactions," in *Proceedings of ICWRS 2014, June 2014* (Bologna: IAHS Publications), 313–319.

Arnal, L., Cloke, H. L., Stephens, E., Wetterhall, F., Prudhomme, C., Neumann, J., et al. (2017). Skilful seasonal forecasts of streamflow over Europe? *Hydrol. Earth Syst. Sci.* 22, 2057–2072. doi: 10.5194/hess-2017-610

Barnett, T. P., Adam, J. C., and Lettenmaier, D. P. (2005). Potential impacts of a warming climate on water availability in snow-dominated regions. *Nature* 438:303–309. doi: 10.1038/nature04141

Bergstrand, M., Asp, S., and Lindström, G. (2014). Nationwide hydrological statistics for Sweden with high resolution using the hydrological model S-HYPE. *Hydrol. Res.* 45, 349–356. doi: 10.2166/nh.2013.010

Bunn, S. E., and Arthington A. H. (2002). Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environ. Manage.* 30, 492–507. doi: 10.1007/s00267-002-2737-0

Chow, V. T., Maidmeni, D. R., and Ma, L. W. (1988). *Applied Hydrology. McGraw-Hill Series in Water Resources and Environmental Engineering.* Berkshire: McGraw-Hill Book Co (UK) Ltd.

Elmhagen, B., Destouni, G., Angerbjörn, A., and Borgström, S., Boyd, E., et al. (2015). Interacting effects of change in climate, human population, land use, and water use on biodiversity and ecosystem services. *Ecol. Soc.* 20, 1–10. doi: 10.5751/ES-07145-200123

Falkenmark, M., and Molden, D. (2008). Wake up to realities of river basin closure. *Water Resour. Dev*. 24, 201–215. doi: 10.1080/07900620701723570

Foster, K., Bertacchi Uvo, C., and Olsson, J. (2017). The development and evaluation of a hydrological seasonal forecast system prototype for predicting spring flood volumes in Swedish rivers. *Hydrol. Earth Syst. Sci.* 22, 2953–2970. doi: 10.5194/hess-2017-588

Godsey, S. E., Kirchner, J. W., and Tague, C. L. (2013). Effects of changes in winter snowpacks on summer low flows: case studies in the Sierra Nevada, California, USA. *Hydrol. Process.* 28, 5048–5064. doi: 10.1002/hyp.9943

Grafton, R. Q., Pittock, J., Davis, R., Williams, J., Fu, G., Warburton, M., et al. (2013). Global insights into water resources, climate change and governance. *Nat. Clim. Change* 3, 315–321. doi: 10.1038/nclimate1746

Hedström-Ringvall, A., Kjörk, C., Pettersson, K., Engström, M., Ljung, P., Hjerdt, N., et al. (2017b). *Dalälvens Vattenkraftssystem*. Report from Länsstyrelsen i Dalarnas län, No. 2017:03, ISSN: 1654-7691, 186 pages (in Swedish).

Hedström-Ringvall, A., Kjörk, C., Pettersson, K., Engström, M., Wisaeus, D., Hjerdt, N., et al. (2017a). Ekologiskt anpassad årsreglering av Dalälven–Naturvårdsnytta och påverkan på vattenkraftssystemet, Report from Länsstyrelsen i Dalarnas län, No. 2017:09, ISSN: 1654-7691, 166 pages (in Swedish). Available online at http://www.lansstyrelsen.se/Dalarna/Sv/publikationer/rapporter-2017/Pages/2017-09.aspx

Johansson, B. (2002). *Estimation of Areal Precipitation for Hydrological Modelling in Sweden*. PhD Thesis, Earth Sciences Centre, Dept Phys. Geog., Göteborg University, Sweden.

Krasting, J. P., Broccoli, A. J., Dixon, K., and Lanzante, J. (2013). Future changes in northern hemisphere snowfall. *J. Clim*. 26, 7813–7828. doi: 10.1175/JCLI-D-12-00832.1

KSLA (2015). *Forests and Forestry in Sweden*. The Royal Swedish Academy of Agriculture and Forestry (KSLA). Available online at: http://www.ksla.se/wp-content/uploads/2015/08/Forests-and-Forestry-in-Sweden_2015.pdf (in Swedish)

Laudon, H., Sponseller, R. A., Lucas, R. W., Futter, M. N., Egnell, G., et al. (2011). Consequences of more intensive forestry for the sustainable management of forest soils and waters. *Forests* 2, 243–260. doi: 10.3390/f2010243

Leira, M., and Cantonati, M. (2008). Effects of water-level fluctuations on lakes: an annotated bibliography. *Hydrobiologia* 613, 171–184. doi: 10.1007/s10750-008-9465-2

Lindström, G., Pers, C., Rosberg, J., Strömqvist, J., and Arheimer, B. (2010). Development and testing of the HYPE (Hydrological Predictions for the Environment) water quality model for different spatial scales. *Hydrol. Res.* 41, 295–319. doi: 10.2166/nh.2010.007

Lytle, D. A., and Poff, N. L. (2004). Adaptation to natural flow regimes. *Trends Ecol. Evol.* 19, 94–100. doi: 10.1016/j.tree.2003.10.002

Molini, A., Katul, G. G., and Porporato, A. (2011). Maximum discharge from snowmelt in a changing climate. *Geophys. Res. Lett.* 38:L05402. doi: 10.1029/2010 GL046477

Nilsson, C., Reidy, C. A., Dynesius, M., and Revenga, C. (2005). Fragmentation and flow regulation of the world's large river systems. *Science* 308, 405–408. doi: 10.1126/science.1107887

Nilsson, P., Cory, N., and Wikberg, P.-E. (2016). *Skogsdata 2016: Aktuella Uppgifter om de Svenska Skogarna från Riksskogstaxeringen, Tema: Skogen då, nu Och i Framtiden*. Umeå: Institutionen för skoglig resurshushållning, Sveriges lantbruksuniversitet, SLU. Available online at: http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-e-3484 (in Swedish)

Palmer, M. A., Lettenmaier, D. P., Poff, N. L., Postel, S. L., Richter, B., and Warner, R. (2009). Climate change and river ecosystems: protection and adaptation options. *Environ. Manage.* 44, 1053–1068. doi: 10.1007/s00267-009-9329-1

Palmer, M. A., Reidy Liermann, C. A., Nilsson, C., Flörke, M., Alcamo, J., Lake, P. S., et al. (2008). Climate change and the world's river basins: anticipating management options. *Front. Ecol. Environ.* 6, 81–89. doi: 10.1890/060148

Rheinheimer, D. E., and Viers, J. H. (2015). Combined effects of reservoir operations and climate warming on the flow regime of hydropower bypass reaches of California's Sierra Nevada. *River Res. Appl.* 31, 269–279. doi: 10.1002/rra.2749

Samuelsson, P., Jones, C. G., Willén, U., Ullerstig, A., Gollvik, S., Hansson, U., et al. (2011). The Rossby centre regional climate model RCA3: model description and performance. *Tellus A* 63, 4–23. doi: 10.1111/j.1600-0870.2010.00478.x

Strömqvist, J., Arheimer, B., Dahné, J., Donnelly, C., and Lindström, G. (2012). Water and nutrient predictions in ungauged basins: set-up and evaluation of a model at the national scale. *Hydrol. Sci. J.* 57, 229–247. doi: 10.1080/02626667.2011.637497

Yang, W., Andréasson, J., Graham, L. P., Olsson, J., Rosberg, J., and Wetterhall, F. (2010). Distribution based scaling to improve usability of regional climate model projections for hydrological climate change impacts studies. *Hydrol. Res.* 41, 211–229. doi: 10.2166/nh.2010.004

Zinke, P. (2013). *Application of the Building Block Methodology to the Dalälven Project*. Länsstyrelsen Gävleborg, Report No. 2013:11. Available online at http://www.lansstyrelsen.se/Gavleborg/SiteCollectionDocuments/Sv/publikationer/2013/RLS_rapporter_Bilagor/Bilaga15_Konekvens_reglering.pdf

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Plant Science

ORIGINAL RESEARCH
published: 14 September 2017
doi: 10.3389/fpls.2017.01546

Elevated CO_2 Increases Nitrogen Fixation at the Reproductive Phase Contributing to Various Yield Responses of Soybean Cultivars

Yansheng Li[1], Zhenhua Yu[1], Xiaobing Liu[1], Ulrike Mathesius[2], Guanghua Wang[1], Caixian Tang[3], Junjiang Wu[4], Judong Liu[1], Shaoqing Zhang[1] and Jian Jin[1,3]*

[1] Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin, China, [2] Division of Plant Science, Research School of Biology, Australian National University, Canberra, ACT, Australia, [3] Centre for AgriBioscience, La Trobe University, Bundoora, VIC, Australia, [4] Key Laboratory of Soybean Cultivation of Ministry of Agriculture, Soybean Research Institute, Heilongjiang Academy of Agricultural Sciences, Harbin, China

Edited by:
José Manuel Mirás-Avalos,
Centro de Edafología y Biología
Aplicada del Segura (CSIC), Spain

Reviewed by:
Mauro Centritto,
Trees and Timber Institute (CNR), Italy
Fernando José Cebola Lidon,
Faculdade de Ciências e Tecnologia
da Universidade Nova de Lisboa,
Portugal

***Correspondence:**
Jian Jin
j.jin@latrobe.edu.au

Specialty section:
This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Plant Science

Received: 06 May 2017
Accepted: 23 August 2017
Published: 14 September 2017

Citation:
Li Y, Yu Z, Liu X, Mathesius U,
Wang G, Tang C, Wu J, Liu J, Zhang S
and Jin J (2017) Elevated CO_2
Increases Nitrogen Fixation at the
Reproductive Phase Contributing to
Various Yield Responses of Soybean
Cultivars. Front. Plant Sci. 8:1546.
doi: 10.3389/fpls.2017.01546

Nitrogen deficiency limits crop performance under elevated CO_2 (eCO_2), depending on the ability of plant N uptake. However, the dynamics and redistribution of N_2 fixation, and fertilizer and soil N use in legumes under eCO_2 have been little studied. Such an investigation is essential to improve the adaptability of legumes to climate change. We took advantage of genotype-specific responses of soybean to increased CO_2 to test which N-uptake phenotypes are most strongly related to enhanced yield. Eight soybean cultivars were grown in open-top chambers with either 390 ppm (aCO_2) or 550 ppm CO_2 (eCO_2). The plants were supplied with 100 mg N kg^{-1} soil as ^{15}N-labeled calcium nitrate, and harvested at the initial seed-filling (R5) and full-mature (R8) stages. Increased yield in response to eCO_2 correlated highly ($r = 0.95$) with an increase in symbiotically fixed N during the R5 to R8 stage. In contrast, eCO_2 only led to small increases in the uptake of fertilizer-derived and soil-derived N during R5 to R8, and these increases did not correlate with enhanced yield. Elevated CO_2 also decreased the proportion of seed N redistributed from shoot to seeds, and this decrease strongly correlated with increased yield. Moreover, the total N uptake was associated with increases in fixed-N per nodule in response to eCO_2, but not with changes in nodule biomass, nodule density, or root length.

Keywords: open-top chamber, ^{15}N labeling, nodule density, symbiotic N_2 fixation, N remobilization, *Glycine max* L.

INTRODUCTION

Plant demand for nitrogen (N) likely increases under elevated atmospheric CO_2 (eCO_2). Nitrogen addition enhances CO_2 effects on plant productivity. In ryegrass swards, compared to non-N control, N addition resulted in a greater yield response to eCO_2 (Schneider et al., 2004). Moreover, eCO_2 significantly increased N uptake of wheat (Butterly et al., 2016). It appears that sufficient N supply may lead to optimization of photosynthetic processes to favor the productivity under eCO_2 (Ainsworth and Long, 2005; Luo et al., 2006; Langley and Megonigal, 2010).

Therefore, the magnitude of response in plant productivity largely depends on how plant N uptake is capable to keep pace with eCO_2-induced stimulation of carbohydrate production and growth. Plants may positively regulate a series of physiological processes, such as secretion of enzymes and root growth, to increase the capacity of plant nutrient acquisition for optimal adaptability to eCO_2 (Rogers et al., 2006; Sardans and Peñuelas, 2012). In legumes, symbiotic N_2 fixation has been considered as the most influential factor affecting plant N uptake and productivity under eCO_2 (Ainsworth et al., 2003). Elevated CO_2 increased nodule size and number, specific nitrogenase activity and plant N content, and consequently increased biomass and/or seed yield in legumes such as *Trifolium repens*, *Lupinus albus*, *Pisum sativum,* and *Glycine max* (Zanetti et al., 1996, 1997; Lee et al., 2003; Rogers et al., 2009; Butterly et al., 2016). However, the responses of symbiotic N_2 fixation to eCO_2 may vary between legume species and even varieties within a given species. For example, Lam et al. (2012) reported that eCO_2 (550 ppm) significantly increased the amount of symbiotic N_2 fixation in the soybean (*G. max*) cultivar Zhonghuang 13 but had no effect in Zhonghuang 35.

Labile N in soil is an important source to satisfy plant N demand under eCO_2 (Shimono and Bunce, 2009). Studies have shown that the increased root biomass of crops grown under eCO_2 could increase N uptake from soil (Matamala and Schlesinger, 2000; Bertrand et al., 2007). Moreover, Matamala et al. (2003) reported that under eCO_2, fine roots are more important for N uptake than total root biomass. However, to our knowledge, the extent of N originating from N_2 fixation and soil/fertilizer among the soybean cultivars in response to eCO_2 has not been quantified, especially in Mollisol regions where soybean is a major crop (Liu and Herbert, 2002; Yu et al., 2016). Investigating the cultivar variation in N uptake in response to eCO_2 is essential to predict the adaptability of soybean cultivars and formulate the N fertilization strategy to increase N-use efficiency in the future.

Besides plant N uptake, the remobilization of N from vegetative to reproductive sinks during the reproductive stages of crop development is an important contributor to maximizing yield in soybean. Because N previously accumulated in vegetative organs can be remobilized to seeds when exogenous N cannot fulfill the N demand in seed filling (Salon et al., 2001; Schiltz et al., 2005), the effect of eCO_2 on the dynamics of N accumulation might determine the pattern of N remobilization. It has been reported that the extent of the contribution of N remobilization to seed N varies from 80 to 90% in soybean cultivars (Warembourg and Fernandez, 1985; Kinugasa et al., 2012). However, few studies have investigated the N remobilization of soybean cultivars in response to eCO_2.

Therefore, N uptake and its partitioning in plants under eCO_2 are important characteristics of phenotypic plasticity in response to climate change. While most previous studies have focused on responses in single genotypes, or compared different unrelated species, our study utilized a group of soybean genotypes that differed in their plastic responses to eCO_2. Using the ^{15}N dilution method (Unkovich and Baldock, 2008), we aimed to assess the effect of eCO_2 on the origins of plant N, i.e., symbiotically fixed-N, fertilizer N, and soil N, and the correspondent N remobilization during the seed-filling stage. We then correlated these changes with yield stimulation under eCO_2. We hypothesized that eCO_2 would increase N_2 fixation and alter distribution of the fixed-N to seed to contribute to yield gain.

MATERIALS AND METHODS

Research Site and Experimental Design

A pot experiment was conducted in open-top chambers (OTC) at the Northeast Institute of Geography and Agroecology (45°73′N, 126°61′E), Chinese Academy of Sciences, Harbin, China. The experiment had a random block design comprising two atmospheric CO_2 concentration levels and eight soybean cultivars with three replications. The two CO_2 levels were ambient CO_2 (aCO_2; 390 ppm) and eCO_2 (550 ppm). Each couple of OTC (one per CO_2 treatment) was considered as a block, and they were randomly located in the field site. The eight soybean cultivars were Xiaohuangjin (XHJ, released in 1951), Hejiao 6 (HJ6, released in 1962), Nenfeng 1 (NF1, released in 1972), Nenfeng 9 (NF9, released in 1980), Suinong 8 (SN8, released in 1989), Suinong 14 (SN14, released in 1996), Heinong 45 (HN45, released in 2003), Suinong 22 (SN22, released in 2005). These cultivars have been widely grown in northeast China with a growing area of more than 2 million ha (Jin et al., 2012).

Six octagonal OTC (three for each CO_2 concentration) were constructed with a steel frame. The main body of each OTC is 3.5 m in diameter, 2.0 m high and with a 0.5-m high canopy, which formed a 45° angle with the plane (Zhang et al., 2014). The OTC were covered with polyethylene film (transparency $\geq$ 95%). This OTC design has been widely used in CO_2-associated studies (e.g., Liu et al., 2016; Yu et al., 2016; Chaturvedi et al., 2017). A digital CO_2-regulating system (Beijing VK2010, China) was installed to monitor the CO_2 level in each OTC and automatically regulate the supply of CO_2 gas (99.9%) to achieve CO_2 concentrations of 550 ± 30 ppm for eCO_2 and 390 ± 30 ppm for aCO_2. There were 16 pots per OTC with two pots per cultivar for two harvest time points.

Plant Growth and ^{15}N Labeling

The soil used in this study was classified as a Mollisol, and had an organic C content of 28.3 mg g^{-1} soil, total N of 2.24 mg g^{-1} soil, available N of 260 $\mu g\ g^{-1}$ soil, and a pH of 6.97 (1:5 H_2O). Nitrogen fertilizer was applied as $Ca(NO_3)_2$ with 5% of ^{15}N atom excess at a rate of 100 mg N kg^{-1} soil. The procedure of ^{15}N labeling is described in Li et al. (2016).

Before sowing, uniform seeds were selected and germinated at 25°C on moistened filter paper. After 2-day germination, six seeds were sown in each pot (20 cm diameter and 40 cm high) containing 9 L soil and thinned to 2 plants 10 days after emergence. Thus, there were six pots per cultivar grown in either aCO_2 or eCO_2 environment. The pot design was considered appropriate for precise isotope labeling and root sampling (Ainsworth et al., 2002). However, the pot size used in this experiment might limit, to some extent, the plant response to CO_2 elevation as Arp (1991) stated that plants grown in pots of 3.5–12.5 L had intermediate responses to eCO_2. Soil water content was maintained at 80 ± 5% of field capacity by weighing and watering. In addition, wheat (*Triticum aestivum* L. cv. Longmai 26) plants were grown under the same conditions as non-N_2 fixing reference species (Rennie and Dubetz, 1986) due to lack of suitable non-nodulating isolines, and was harvested at physiological maturity. Although choosing wheat as non-fixing control exhibits some methodological limitations (Unkovich and Baldock, 2008), wheat has been widely used as a reference plant species in many studies to estimate legume N_2 fixation (Rennie and Dubetz, 1986; Carranca et al., 1999; Lam et al., 2012).

Harvest and Measurements

Plants of three pots were harvested at the R5 (beginning seed formation, 81 days after sowing) and R8 stages (maturity, 120 days after sowing), respectively (Fehr et al., 1971). Shoots were cut at the cotyledon node level and separated into stems plus petioles, leaves and pods at R5, and additionally seeds at R8. The abscised leaves in each pot between R5 and R8 stages were collected for C and N measurements. The entire root system of each plant was carefully separated from soil, and then washed with tap water to remove soil particles adhering to the roots. Nodules were removed from the root system, counted and weighed. The root length and diameter classes of roots were then determined using WinRhizo 2004b (Régent Instruments Inc., Québec, Canada). According to their diameter, roots were classified as fine roots (<0.5 mm), intermediate roots (0.5–1.0 mm), and coarse roots (>1 mm) (Costa et al., 2002).

All plant samples were dried at 70°C for 72 h, and then finely ground in a ball mill (Retsol MM2000, Retsch, Haan, Germany). The $^{15}N/^{14}N$ ratio of all samples was measured with an isotope ratio mass spectrometer (Delta^{plus}, Finnigan MAT GmbH, Bremen, Germany). The C and N contents of plant samples were determined using an ELEMENTAR III analyzer (Hanau, Germany).

Calculations and Statistical Analysis

Atom% ^{15}N excess was calculated with reference to the natural ^{15}N abundance in the atmosphere (0.3663 atom% ^{15}N; Mariotti et al., 1984). The percentage of plant N derived from N_2 fixation (%Ndfa) was calculated as follows (Rennie and Dubetz, 1986):

$\%\text{Ndfa} = \{1 - [\text{atom}\%\ ^{15}\text{N excess } (fs)/\text{atom}\%\ ^{15}\text{N excess } (nfs)]\} \times 100$

where *fs* and *nfs* represented fixing and non-fixing (wheat) system, respectively.

N_2 fixed was calculated as follows:

$N_2 \text{ fixed (mg plant}^{-1}) = (\%\text{Ndfa}/100) \times N_{plant}(\text{mg plant}^{-1})$

where N_{plant} was the N content of each plant compartment.

The amounts of plant N derived from fertilizer (Ndff_{plant}) and soil (Ndfs_{plant}) were estimated (Martínez-Alcántara et al., 2012) as follows:

$\text{Ndff}_{plant} = N_{plant}(\text{mg plant}^{-1}) \times \text{N atom}\%\ ^{15}\text{N excess in plant/N atom}\%$

^{15}N excess in fertilizer (19.83%)

$\text{Ndfs}_{plant} = N_{plant}(\text{mg plant}^{-1}) - \text{Ndff}_{plant} - N_2 \text{ fixed}$

The amount of N remobilized from vegetative organs to seeds was estimated as N content in vegetative organs aboveground at R5 subtracted from that at R8 (Egli et al., 1978). Nodule density was calculated as nodule number divided by total root length. Two-way ANOVA on variables including yield components, parameters of plant N, root morphology, nodule number, and nodule fresh weight was performed with Genstat 13 (VSN International, Hemel Hemspstead, UK). Partial correlation analyses were used to evaluate the correlations of N assimilation indices with nodule characteristics, root morphology and yield gain in response to eCO_2 (Peng et al., 2004). The least significance

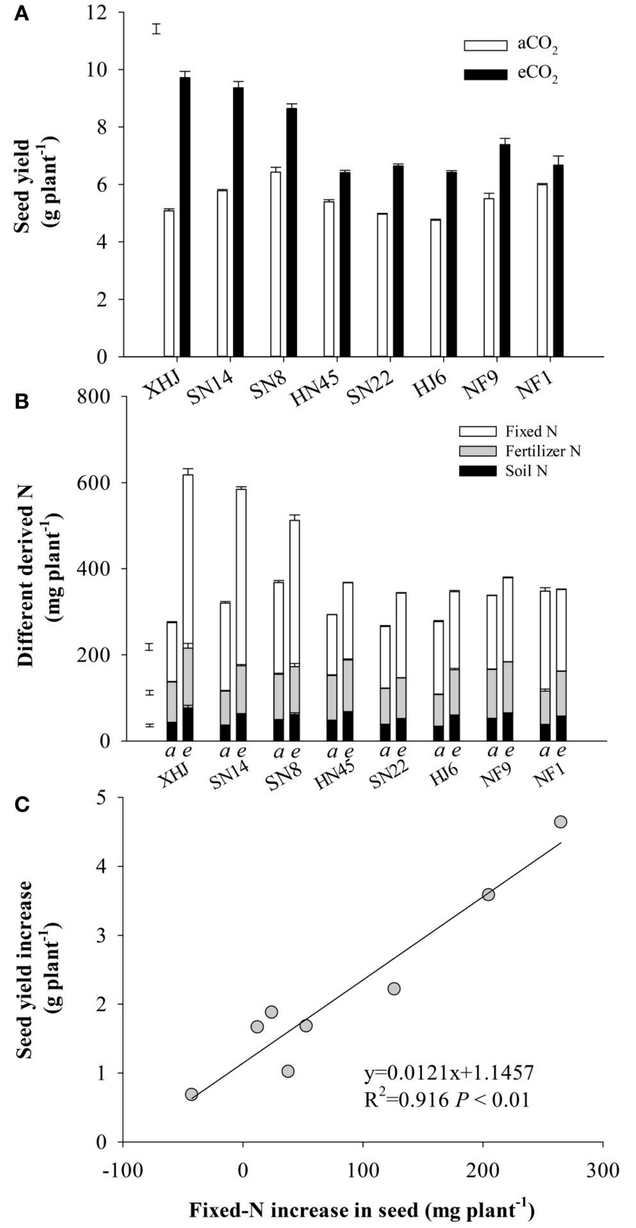

FIGURE 1 | (A) Seed yield, **(B)** soil-derived N, fertilizer-derived N, and symbiotically fixed-N content in seed and **(C)** the relationship between increased fixed-N in seeds and yield increment of eight soybean cultivars under eCO_2 relative to aCO_2. Each data point represents one cultivar. The error bars represent standard error, and separate vertical bar(s) in **(A)** and **(B)** indicate the LSD (P < 0.05) for the CO_2 × cultivar interaction.

difference (LSD) was used to assess the differences among treatments at $P = 0.05$.

RESULTS

Seed Yield and Seed N Origins

Compared to aCO_2, eCO_2 increased seed yield by an average of 40% (**Figure 1A**). The yield response to eCO_2 varied among cultivars ($P < 0.001$), resulting in a 91% increase in XHJ in comparison to 12% in NF1, and leading to a significant $CO_2 \times$ cultivar interaction ($P < 0.001$). Interestingly, the cultivars showing the highest yield under eCO_2 were not the ones showing the highest yield under aCO_2, but exhibited the biggest increase in yield gain. In addition, the N content of the seed showed a shift in origin toward greater fixed N under eCO_2 (**Figure 1B**). Overall, there was a strong ($P < 0.001$) correlation between the increase in fixed-N content of seeds and their yield increase under eCO_2 (**Figure 1C**).

Shoot Biomass and N Content

Shoot biomass at R8 also significantly increased (by 46% on average) under eCO_2 compared with aCO_2 (Figure S1) with a minimum increase of 22% for HN45 and a maximum of 87% for XHJ ($P < 0.001$). Compared with aCO_2, eCO_2 increased shoot N content by 11% at R5, and 41% at R8 ($P < 0.05$) (**Table 1**). Among cultivars, the largest increase in shoot N content at R8 in response to eCO_2 was observed in XHJ (119%), and the smallest one (7%) in NF1.

Elevated CO_2 decreased shoot N concentration (mg g^{-1}) by an average of 30% at R5 (Figure S1). At R8, eCO_2 did not affect shoot N concentration in SN8, SN14, HN45, and SN22 (Figure S1), but increased it by 17% in XHJ.

Shoot N Origins

Compared to aCO_2, eCO_2 decreased the fixed-N content (mg $plant^{-1}$) of the shoot at R5 ($P < 0.05$), but significantly increased it at R8 (**Table 1**). The maximum increase was found in XHJ

TABLE 1 | Shoot N content, symbiotically fixed-N (SNF) content, fertilizer-derived, and soil-derived N content in shoot of eight soybean cultivars grown under aCO_2 or eCO_2 till R5 (81 days after sowing) and R8 (120 days after sowing).

	N content (mg $plant^{-1}$)		SNF N content (mg $plant^{-1}$)		Fertilizer N content (mg $plant^{-1}$)		Soil N content (mg $plant^{-1}$)	
	aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2
R5 (81 DAYS AFTER SOWING)								
XHJ	220	262*	79.0	79.2^{ns}	96.6	118*	44.4	64.8*
SN14	216	233^{ns}	80.5	64.6*	93.4	107*	42.9	58.9*
SN8	240	266^{ns}	89.9	59.3*	115	118^{ns}	52.9	65.2*
HN45	258	243*	74.5	62.5*	95.1	112*	43.7	61.7*
SN22	217	231*	78.8	56.4*	79.1	106*	36.4	58.7*
HJ6	218	264*	93.5	80.7*	85.3	118*	39.2	65.0*
NF9	213	236*	83.3	67.6*	107	128*	49.2	70.5*
NF1	194	222^{ns}	76.6	61.2*	95.5	111*	43.9	61.2*
$LSD_{0.05}$	19.6		7.76		9.16		4.64	
SIGNIFICANCE LEVEL								
CO_2	<0.001		<0.001		<0.001		<0.001	
Cultivar	<0.001		<0.001		<0.001		<0.001	
$CO_2 \times$Cultivar	0.004		<0.001		0.004		0.009	
R8 (120 DAYS AFTER SOWING)								
XHJ	339	742*	162	466*	114	167*	63.6	110*
SN14	405	680*	249	461*	97.1	127*	59.1	91.6*
SN8	448	626*	248	399*	126	134^{ns}	73.7	93.9*
HN45	356	461*	174	233*	116	133	66.5	94.7*
SN22	329	423*	169	231*	102	112*	58.3	79.0*
HJ6	349	438*	201	223*	91.8	125*	55.5	90.0*
NF9	418	466*	215	241*	128	130^{ns}	74.7	94.8*
NF1	415	442*	261	234^{ns}	95.9	121*	57.8	87.6*
$LSD_{0.05}$	22.4		16.0		12.4		7.31	
SIGNIFICANCE LEVEL								
CO_2	<0.001		<0.001		<0.001		<0.001	
Cultivar	<0.001		<0.001		<0.001		<0.001	
$CO_2 \times$Cultivar	<0.001		<0.001		<0.001		<0.001	

** and ns indicate significant and non-significant difference (t-test) between aCO_2 and eCO_2, respectively, for individual cultivars. LSD values correspond to the $CO_2 \times$ cultivar interaction (two-way ANOVA).*

(188%) while no difference occurred in NF1 ($P > 0.05$) at R8 (**Table 1**).

Elevated CO_2 increased the accumulation of the fertilizer-derived N in the shoot (mg $plant^{-1}$) by 20 and 21% at R5 and R8, respectively (**Table 1**). The extent of increase of fertilizer-derived N under eCO_2 differed among cultivars. At R5, the increase in fertilizer-derived N in HJ6 under eCO_2 reached 38% compared to aCO_2, while there was no CO_2 effect in SN8. At R8, eCO_2 increased fertilizer-derived N by 46% in XHJ but did not affect it in SN8 and NF9. A significant ($P < 0.001$) $CO_2\times$ cultivar interaction was observed at R5 and R8 (**Table 1**).

Similarly, eCO_2 increased the soil-derived N accumulation in the shoot by 45 and 47% at R5 and R8, respectively (**Table 1**). A significant $CO_2\times$ cultivar interaction on soil-derived N content in the shoot was observed (**Table 1**). At R5, soil-derived N content increased by 66% in HJ6 under eCO_2 in comparison to 23% in SN8. At R8, XHJ exhibited 73% increase for soil-derived N content, but only 27% increase in SN8 and NF1 was observed. However, overall, there was no significant correlation between yield gain and either soil-derived or fertilizer-derived N uptake under eCO_2 (Figure S2).

Under eCO_2, the proportion of fixed-N in the shoot at R5 decreased ($P < 0.05$) by 27% compared to aCO_2 (**Figure 2A**). In contrast, the proportion of fertilizer- and soil-derived N in the shoot at R5 increased by 9.1 and 31%, respectively, under eCO_2. At R8, however, eCO_2 increased the proportion of fixed-N in the shoot of all cultivars except for HJ6 (−12%) and NF1 (−16%) (**Figure 2B**). Under eCO_2, the proportion of fertilizer-derived N decreased in all cultivars. Elevated CO_2 decreased the proportion of soil-derived N in the shoot of XHJ, SN8, and SN14, but increased it in HJ6, NF1, NF9, HN45, and SN22, leading to significant $CO_2\times$ cultivar interactions (**Figure 2B**).

N Remobilization

Elevated CO_2 significantly decreased the proportion of the remobilized N in seeds, with the greatest decrease for XHJ and no significant response for HJ6, NF9, and NF1 (**Figure 3A**).

Approximately 68% of N was remobilized from vegetative organs to seeds at aCO_2 in comparison to 60% under eCO_2 (**Figure 3B**). Elevated CO_2 significantly ($P < 0.05$) decreased the proportion of the N remobilization in XHJ, NF1, SN8, and HF45, but did not affect it in HJ6, NF9, SN14, and SN22, contributing to a significant $CO_2\times$ cultivar interaction.

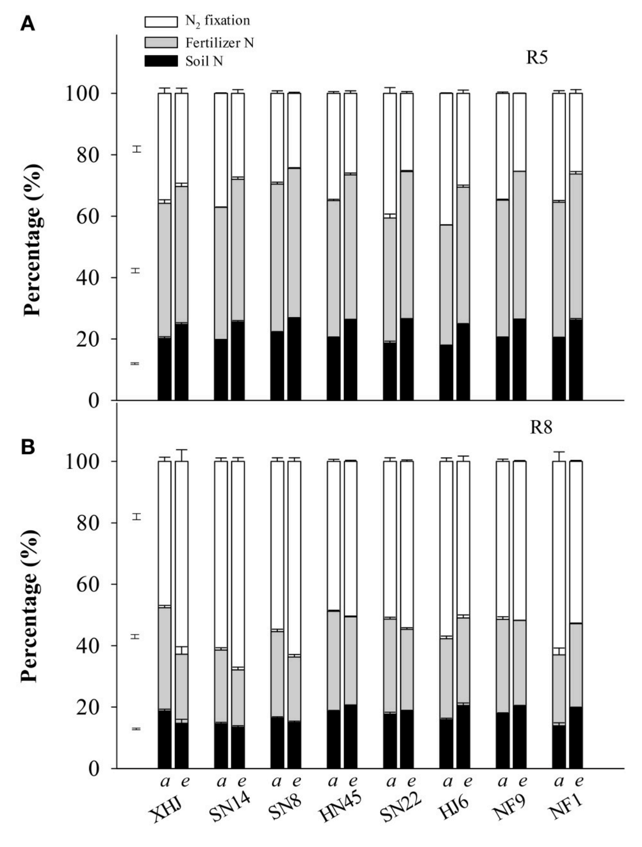

FIGURE 2 | The effect of CO_2 on the percentage of plant N derived from symbiotically fixed-, fertilizer-, and soil-N at **(A)** R5 and **(B)** R8 (81 and 120 days after sowing, respectively). The error bars represent standard error, and separate vertical bars indicate the LSD ($P < 0.05$) for the CO_2 × cultivar interaction. The letters of *a* and *e* on the x-axis indicate aCO_2 and eCO_2, respectively.

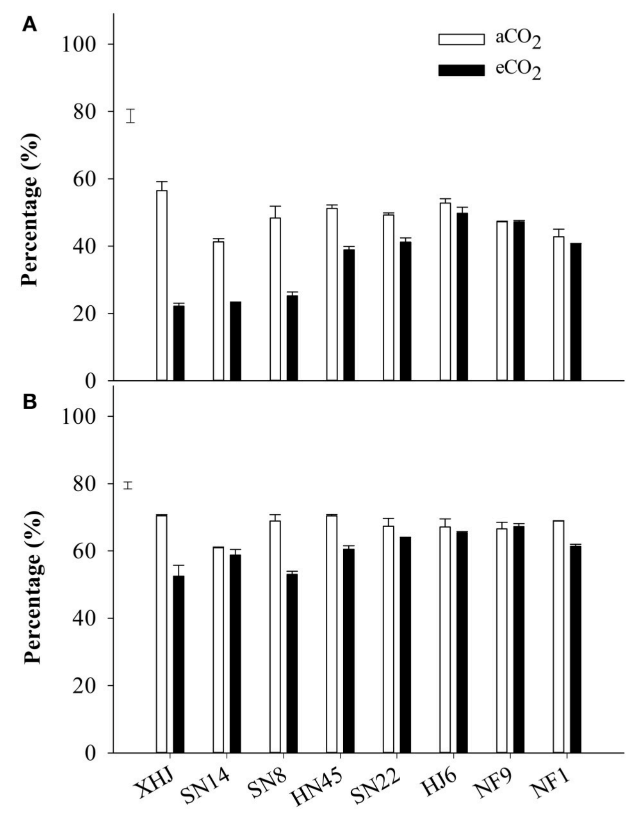

FIGURE 3 | The percentage of **(A)** remobilized N in seed and **(B)** the percentage of N remobilized from vegetative organs to seeds of eight cultivars grown under aCO_2 or eCO_2. The error bars represent standard error, and the separate vertical bar in each panel indicates the LSD ($P < 0.05$) for the CO_2 × cultivar interaction.

Relationship between Yield and N

The stimulation of fixed-N was significantly correlated with seed N increase (**Figure 4A**) and yield gain (**Figure 4B**), while the decrease of remobilized N to seed significantly correlated with the response of seed N to eCO_2 (**Figure 5A**) and yield (**Figure 5B**). No significant correlation ($P > 0.05$) was found between the increase in fertilizer- or soil-derived N content and the increase of yield in response to eCO_2 (Figure S3).

Root Morphology

Elevated CO_2 increased total root length ($P < 0.05$) by an average of 19% (Table S1). The length of fine roots accounted for more than 85% of total root length, and fine roots (<0.5 mm) had a positive ($P < 0.05$) growth response to eCO_2 in all cultivars except for SN22 (Table S1). Only the length of intermediate roots of XHJ, and the length of coarse roots of SN22 and NF1 were significantly higher under eCO_2 than under aCO_2 ($P < 0.05$).

Elevated CO_2 significantly increased the N uptake per unit of root length in XHJ, SN14, HN45, SN22, and NF1 compared to aCO_2 ($P < 0.05$), but did not in SN8, HJ6, and NF9 (Table S2). The fertilizer-derived N uptake per unit of root length did not significantly change in response to eCO_2 except for NF1 (+15%) and NF9 (–12%) ($P < 0.05$). The soil-derived N uptake per unit of root increased by 26% ($P < 0.05$) across the cultivars under eCO_2 compared to aCO_2, with the maximum increase (44%) for XHJ and the minimum (9%) for SN8.

Although there were marked changes in root architecture in response to eCO2, these changes did not directly contribute to yield gain under eCO2. There was no correlation between seed yield increase with changes in total root length, fine, intermediate or coarse root length ($P > 0.05$, Figure S4).

Nodulation

Elevated CO_2 significantly altered the nodule characteristics of soybean. Nodule numbers increased from 79 under aCO_2 to 113 under eCO_2 on average across cultivars (**Table 2**). Nodule number in response to eCO_2 differed among soybean cultivars, with 96% of increase in HJ6 in comparison to only 3% in SN14. A significant ($P < 0.001$). A significant $CO_2\times$ cultivars interaction was observed ($P < 0.001$; **Table 2**). Elevated CO_2 resulted in a significant increase in nodule fresh weight (**Table 2**). The maximum increase (301%) was found in SN14 while the minimum increase was 93% in SN22. Elevated CO_2 significantly increased nodule density of all cultivars but NF9 and SN14 (**Table 2**).

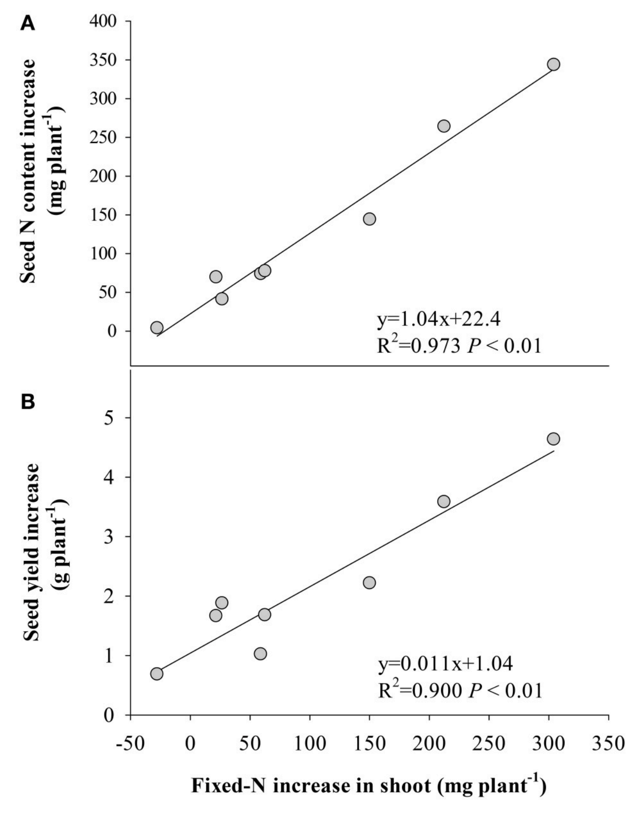

FIGURE 4 | Relationships between the increase in the amount of fixed-N in shoot at R8 (120 days after sowing) and increases in **(A)** seed N and **(B)** seed yield of the eight soybean cultivars under eCO_2 relative to aCO_2. Each point represents one cultivar.

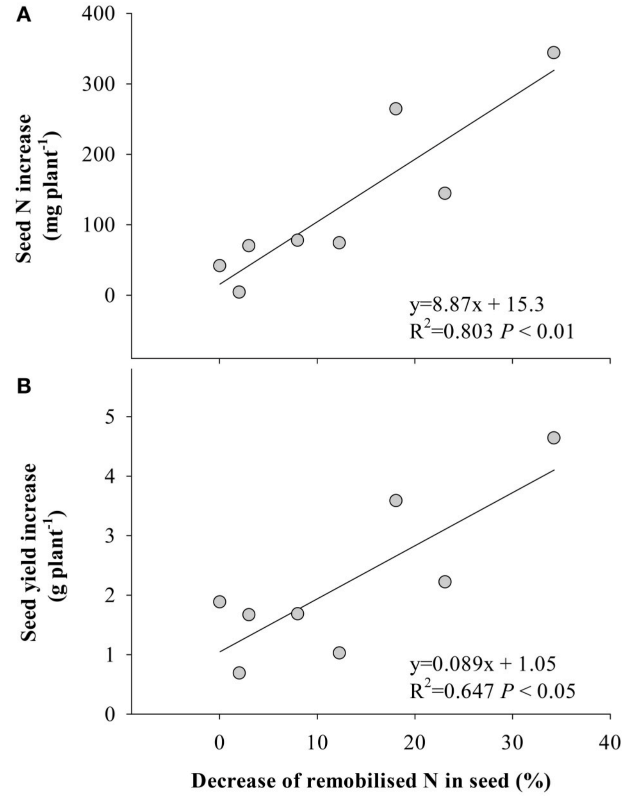

FIGURE 5 | Relationships between the decrease of remobilized N in seed under eCO_2 and **(A)** the seed N content increase, and **(B)** seed yield increase. Each point represents one soybean cultivar. The decrease of remobilized N was calculated as proportion of remobilized N in seed under aCO_2 minus that under eCO_2.

TABLE 2 | Nodule number per plant, nodule fresh weight per plant, nodule density, and single nodule N fixation of eight soybean cultivars grown for 120 days (R8) under aCO_2 or eCO_2.

	Nodule number (number $plant^{-1}$)		Nodule fresh weight (mg $plant^{-1}$)		Nodule density (number m^{-1})		Fixed-N per nodule (mg $nodule^{-1}$)	
	aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2
XHJ	80	132*	368	1,392*	1.35	1.87*	2.04	3.54*
SN14	132	136^{ns}	431	1,726*	2.71	2.27*	1.89	3.42*
SN8	78	112*	554	1,480*	1.19	1.51*	3.19	3.58^{ns}
HN45	101	142*	368	1,035*	1.82	2.32*	1.73	1.65^{ns}
SN22	104	171*	712	1,376*	1.74	2.54*	1.64	1.36^{ns}
HJ6	55	108*	509	1,698*	1.05	1.48*	3.71	2.10*
NF9	56	62*	353	1,125*	1.08	1.03^{ns}	3.83	3.92^{ns}
NF1	30	42*	228	560*	0.52	0.67*	8.97	5.56*
$LSD_{0.05}$	10.3		307		0.15		0.33	
SIGNIFICANCE LEVEL								
CO_2	<0.001		<0.001		<0.001		<0.001	
Cultivar	<0.001		<0.001		<0.001		<0.001	
CO_2 × Cultivar	<0.001		0.002		<0.001		<0.001	

** and ns indicate significant and non-significant difference (t-test) between aCO_2 and eCO_2 within a genotype, respectively, for individual cultivars. LSD values correspond to the CO_2 × cultivar interaction (two-way ANOVA).*

The amount of N fixed per nodule showed different responses to eCO_2 among cultivars (**Table 2**), with 81 and 74% of increase in SN14 and XHJ in comparison to 43 and 38% of reduction in HJ6 and NF1, respectively, resulting in a significant $CO_2 \times$ cultivars interaction ($P < 0.001$).

Irrespective of cultivars, the increase in symbiotically fixed-N content in shoot correlated positively with the increase of fixed-N per nodule in response to eCO_2 ($P < 0.01$; **Figure 6**), but did not correlate with nodule number, fresh weight, and density changes ($P > 0.05$; Figure S4).

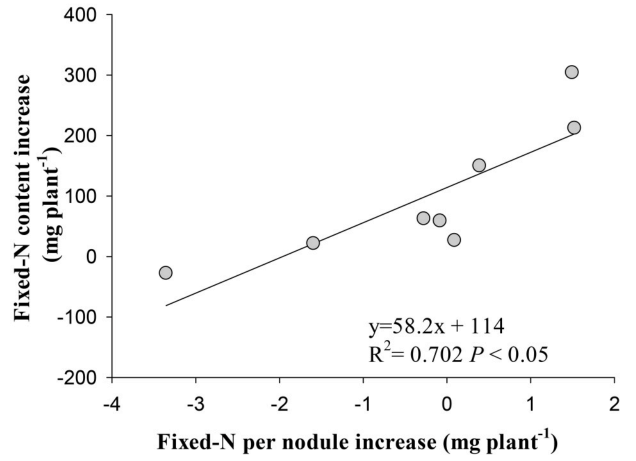

FIGURE 6 | Relationship between the increase in fixed-N per nodule and the increase in fixed-N content in shoot of eight soybean cultivars at R8 (120 days after sowing) under eCO_2 relative to aCO_2. Each point represents one cultivar.

DISCUSSION

This study demonstrated that eCO_2 enhanced total N uptake in soybean, especially during the late reproductive stages. It was evident that the increase in the N content in shoots under eCO_2 was greater at R8 than at R5 (**Table 1**). Moreover, irrespective of cultivars, the extent of the increase in N content derived from symbiotically fixed-N was greater than either fertilizer-derived N or soil-derived N during the period from R5 to R8 (**Table 1**). The fixed-N was the dominant source of plant N, but the proportion of fixed-N was greater under eCO_2 than under aCO_2 (**Figure 2**). The results are consistent with those of previous studies showing that eCO_2 increased total N uptake in agricultural crops (Kimball et al., 2002; Leakey et al., 2009; Jin et al., 2012; Lam et al., 2012; Butterly et al., 2016).

Symbiotic N_2 fixation during this reproductive period is critical for yield gain under eCO_2. This was supported by the positive correlation ($P < 0.05$) between the amount of symbiotically fixed-N and seed yield (**Figure 4**), and the fixed-N being the major source of seed N (**Figure 1**). Furthermore, eCO_2 decreased the proportion of remobilized N in seed (**Figure 3**), indicating that the eCO_2-enhanced total N uptake during the late reproductive stage can largely satisfy N demand in seed development. Since the major source of N remobilization in soybean plants is from leaves (Schiltz et al., 2005; Li et al., 2016), the lesser amount of N removed from vegetative organs including leaves in response to eCO_2 (**Figure 3**) was likely to maintain leaf photosynthetic capacity. Makino and Osmond (1991) also showed that leaf N correlated highly with the photosynthetic function of the leaf. Thus, the maintenance of adequate N in vegetative organs is likely to contribute to the increased biomass accumulation and seed yield under eCO_2 (**Figure 5**).

The stimulation of N_2 fixation during R5 to R8 under eCO_2 was attributed to the increase in nodule N_2 fixation efficiency, as evidenced by the positive correlation between the increase of fixed-N per nodule with the increase in fixed-N content in shoot under eCO_2 (**Figure 6**). In previous studies, eCO_2 enhanced N_2 fixation through increasing specific nitrogenase activity (Saeki et al., 2008). The reason for the increased N_2 fixation is that the enhanced photosynthesis under eCO_2 (Ziska, 2008; Bishop et al., 2015) provides sufficient C sources for maintaining nodule function and N_2 fixation (Li et al., 2016), resulting in the increase in shoot and root biomass (Figure S1). Another reason would be a change of rhizobium community in the rhizosphere of soybean under eCO_2 (Yu et al., 2016), which might favor N_2 fixation efficiency of nodules. This interaction between functional rhizobia and photosynthetic C supply under eCO_2 warrants specific investigation.

A number of studies reported that eCO_2 increased nodule number and biomass in chickpea, field pea (Jin et al., 2012), and common bean (Miyagi et al., 2007; Rogers et al., 2009). In the current study, a similar trend was observed for soybean, but neither the increase of nodule number nor biomass correlated with the increase of fixed-N content (Figure S4). This implies that the increase of fixed-N under eCO_2 cannot be predominantly attributed to the number of nodules.

Elevated CO_2 also changed root morphology with an increase in the proliferation of fine roots, which is likely to enhance plant nutrient absorption (Bentley et al., 2013; Beidler et al., 2015). Fine roots play a key role in N acquisition rather than root biomass (Matamala et al., 2003). In this study, the length of fine roots (<0.5 mm) significantly increased under eCO_2 (Table S1), which helped to increase the uptake of soil and fertilizer N (**Table 1**). This is consistent with previous studies (Mikan et al., 2000; Zak et al., 2000; de Graaff et al., 2006; Beidler et al., 2015). Rogers et al. (1992) suggested that the greater proliferation of roots grown under eCO_2 was a strategy to permit adequate nutrient acquisition under sub-optimal water supply. However, compared to fixed-N, the soil-, and fertilizer-derived N in the plant showed much less response to eCO_2. The increase in fine root growth had no significant correlation with seed yield increase in response to eCO_2 across genotypes (Figure S3), indicating that the contribution of root N uptake to yield gain is minimal under eCO_2. In agreement with our observations, Butterly et al. (2015) also found that N fertilizer did not affect plant N concentration, and the proportion of fertilizer-derived N in field pea decreased under eCO_2.

Nevertheless, eCO_2 increased the uptake of soil N per unit of root length (Table S2). The enhancement of microbial activity and N mineralization in soil under eCO_2 might be the main reason. The growth of fine roots leads to more rhizodeposition, which provides labile C for microorganisms to mineralize more soil organic N (Fischer and Kuzyakov, 2010; Fischer et al., 2010).

The capacity for total N uptake in response to eCO_2 varied among soybean cultivars, XHJ had the greatest increase in N_2 fixation under eCO_2 (**Figure 2**), which supplied a large amount of N to seed during the reproductive stage (**Figure 1B**), and reduced the demand for N remobilization (**Figure 3**). In contrast, NF1 did not exhibit any increase in fixed-N during R5 to R8, and had the least increase in yield under eCO_2 (**Figure 1**). The largest N_2 fixation in XHJ would contribute to a high N_2 fixation efficiency, since the amount of fixed-N per nodule was greatest in this cultivar (**Table 2**). As the dominant rhizobial strains in nodules greatly affected N_2-fixing efficiency (Saeki et al., 2008) and soil microbial communities in the rhizosphere in response to eCO_2 are dependent on soybean cultivars (Yu et al., 2016), the specific interaction between cultivar and rhizobial genera under eCO_2 may influence soybean adaptability to eCO_2. Therefore, the cultivar-specific rhizobia community in nodules may predominantly regulate the N_2-fixing function in response to eCO_2. This hypothesis deserves further research.

In summary, **Figure 7** shows a conceptual diagram illustrating how eCO_2 affects N uptake, and consequent yield gain in

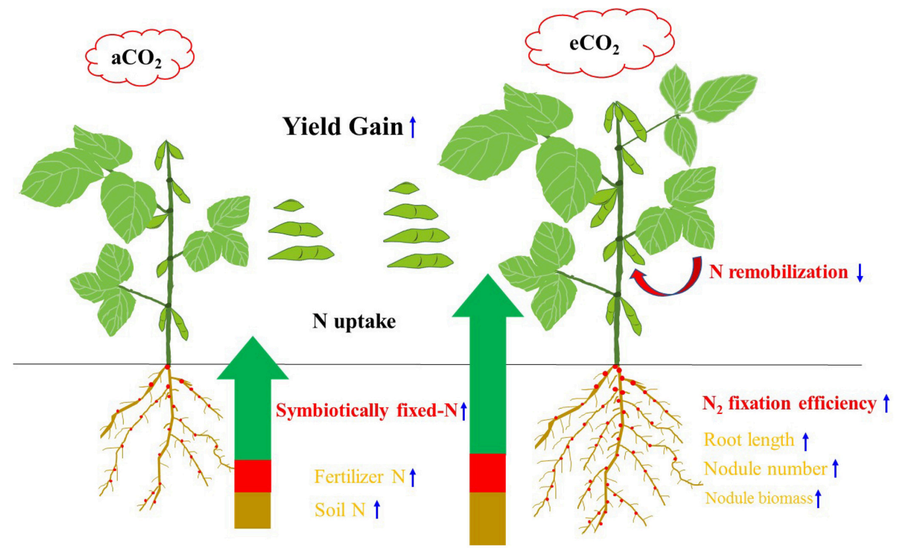

FIGURE 7 | Diagram illustrating the N origins, root morphology, N remobilization, and yield gain of soybean in response to eCO_2. The measurements that were significantly correlated with yield gain ($P < 0.05$) are indicated in red-bold, while the measurements responding to eCO_2 but not correlated with yield gain are shown in orange. Upward and downward arrows indicate increase and decrease under the eCO_2 condition, respectively.

soybean. Elevated CO_2 increased the plants' ability for N uptake. The N_2 fixation during R5 to R8 became a major contributor to the increased N uptake and hence yield gain under eCO_2. The enhanced N_2 fixation under eCO_2 might also lead to the decrease in remobilization of N from vegetative organs, increasing photosynthetic capacity and yield formation. Although eCO_2 facilitated root proliferation and nodule growth, these variables were not correlated with yield gains. Cultivars with a greater N_2-fixing efficiency during the late reproductive phase may exhibit a better adaptability to eCO_2. The specific interaction between cultivar and rhizobia in the rhizosphere of soybean would be the key to this adaptability, and is worth further investigation.

AUTHOR CONTRIBUTIONS

JJ and YL designed the experiments and managed the projects. YL, ZY, JL, SZ, and JW performed experiments. YL, JJ, UM, GW, and CT performed data analysis. JJ, UM, YL, XL, and CT wrote the manuscript.

ACKNOWLEDGMENTS

The project was funded by the National Natural Science Foundation of China (31501259), Heilongjiang Provincial Funds for Distinguished Young Scientists (JC201413), Hundred Talents Program of Chinese Academy of Sciences, the Natural Science Foundation of Heilongjiang Province (QC2015045), and the Foundation of Key Laboratory for Agricultural Environment, Ministry of Agriculture of China.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpls.2017.01546/full#supplementary-material

REFERENCES

Ainsworth, E. A., and Long, S. P. (2005). What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy. *New Phytol.* 165, 351–371. doi: 10.1111/j.1469-8137.2004.01224.x

Ainsworth, E. A., Davey, P. A., Bernacchi, C. J., Dermody, O. C., Heaton, E. A., Moore, D. J., et al. (2002). A meta-analysis of elevated CO_2 effects on soybean (*Glycine max*) physiology, growth and yield. *Global Change Biol.* 8, 695–709. doi: 10.1046/j.1365-2486.2002.00498.x

Ainsworth, E. A., Rogers, A., Blum, H., Nosberger, J., and Long, S. P. (2003). Variation in acclimation of photosynthesis in *Trifolium repens* after eight years of exposure to Free Air CO_2 Enrichment (FACE). *J. Exp. Bot.* 54, 2769–2774. doi: 10.1093/jxb/erg309

Arp, W. J. (1991). Effects of source-sink relations on photosynthetic acclimation to elevated CO_2. *Plant Cell Environ.* 14, 869–875. doi: 10.1111/j.1365-3040.1991.tb01450.x

Beidler, K. V., Taylor, B. N., Strand, A. E., Cooper, E. R., Schonholz, M., and Pritchard, S. G. (2015). Changes in root architecture under elevated concentrations of CO_2 and nitrogen reflect alternate soil exploration strategies. *New Phytol.* 205, 1153–1163. doi: 10.1111/nph.13123

Bentley, L. P., Stegen, J. C., Savage, V. M., Smith, D. D., von Allmen, E. I., Sperry, J. S., et al. (2013). An empirical assessment of tree branching networks and implications for plant allometric scaling models. *Ecol. Lett.* 16, 1069–1078. doi: 10.1111/ele.12127

Bertrand, A., Prevost, D., Bigras, F. J., Lalande, R., Tremblay, G. F., Castonguay, Y., et al. (2007). Alfalfa response to elevated atmospheric CO_2 varies with the symbiotic rhizobial strain. *Plant Soil* 301, 173–187. doi: 10.1007/s11104-007-9436-9

Bishop, K. A., Betzelberger, A. M., Long, S. P., and Ainsworth, E. A. (2015). Is there potential to adapt soybean (*Glycine max* Merr.) to future CO_2? An analysis of the yield response of 18 genotypes in free-air CO_2 enrichment. *Plant Cell Environ.* 38, 1765–1774. doi: 10.1111/pce.12443

Butterly, C. R., Armstrong, R., Chen, D., and Tang, C. (2015). Carbon and nitrogen partitioning of wheat and field pea grown with two nitrogen levels under elevated CO_2. *Plant Soil* 391, 367–382. doi: 10.1007/s11104-015-2441-5

Butterly, C. R., Armstrong, R., Chen, D., and Tang, C. (2016). Free-air CO_2 enrichment (FACE) reduces the inhibitory effect of soil nitrate on N_2 fixation of *Pisum sativum*. *Ann. Bot.* 117, 177–185. doi: 10.1093/aob/mcv140

Carranca, C., de Varennes, A., and Rolston, D. (1999). Biological nitrogen fixation by fababean, pea and chickpea, under field conditions, estimated by the ^{15}N isotope dilution technique. *Eur. J. Agron.* 10, 49–56. doi: 10.1016/S1161-0301(98)00049-5

Chaturvedi, A., Bahuguna, R. N., Pal, M., Shah, D., Maurya, S., and Jagadish, K. S. V. (2017). Elevated CO_2 and heat stress interactions affect grain yield, quality and mineral nutrient composition in rice under field conditions. *Field Crops Res.* 206, 149–157. doi: 10.1016/j.fcr.2017.02.018

Costa, C., Dwyer, L. M., Zhou, X. M., Dutilleul, P., Hamel, C., Reid, L. M., et al. (2002). Root morphology of contrasting maize genotypes. *Agron. J.* 94, 96–101. doi: 10.2134/agronj2002.0096

de Graaff, M. A., van Groenigen, K. J., Six, J., Hungate, B., and van Kessel, C. (2006). Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis. *Global Change Biol.* 12, 2077–2091. doi: 10.1111/j.1365-2486.2006.01240.x

Egli, D. B., Leggett, J. E., and Duncan, W. G. (1978). Influence of N-stress on leaf senescence and N redistribution in soybeans. *Agron. J.* 70, 43–47. doi: 10.2134/agronj1978.00021962007000010011x

Fehr, W. R., Caviness, C. E., Burmood, D. T., and Pennington, J. S. (1971). Stage of development descriptions for soybeans, *Glycine Max* (L.) Merrill. *Crop Sci.* 11, 929–931. doi: 10.2135/cropsci1971.0011183X001100060051x

Fischer, H., and Kuzyakov, Y. (2010). Sorption, microbial uptake and decomposition of acetate in soil: transformations revealed by position-specific ^{14}C labeling. *Soil Biol. Biochem.* 42, 186–192. doi: 10.1016/j.soilbio.2009.10.015

Fischer, H., Eckhardt, K. U., Meyer, A., Neumann, G., Leinweber, P., Fischer, K., et al. (2010). Rhizodeposition of maize: short-term carbon budget and composition. *J. Plant Nutr. Soil Sci.* 173, 67–79. doi: 10.1002/jpln.200800293

Jin, J., Tang, C. X., Armstrong, R., and Sale, P. (2012). Phosphorus supply enhances the response of legumes to elevated CO_2 (FACE) in a phosphorus-deficient vertisol. *Plant Soil* 358, 86–99. doi: 10.1007/s11104-012-1270-z

Kimball, B. A., Kobayashi, K., and Bindi, M. (2002). Responses of agricultural crops to free-air CO_2 enrichment. *Adv. Agron.* 77, 293–368. doi: 10.1016/S0065-2113(02)77017-X

Kinugasa, T., Sato, T., Oikawa, S., and Hirose, T. (2012). Demand and supply of N in seed production of soybean (*Glycine max*) at different N fertilization levels after flowering. *J. Plant Res.* 125, 275–281. doi: 10.1007/s10265-011-0439-5

Lam, S. K., Hao, X. Y., Lin, E. D., Han, X., Norton, R., Mosier, A. R., et al. (2012). Effect of elevated carbon dioxide on growth and nitrogen fixation of two soybean cultivars in northern China. *Biol. Fert. Soils* 48, 603–606. doi: 10.1007/s00374-011-0648-z

Langley, J. A., and Megonigal, J. P. (2010). Ecosystem response to elevated CO_2 levels limited by nitrogen-induced plant species shift. *Nature* 466, 96–99. doi: 10.1038/nature09176

Leakey, A. D. B., Ainsworth, E. A., Bernacchi, C. J., Rogers, A., Long, S. P., and Ort, D. R. (2009). Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *J. Exp. Bot.* 60, 2859–2876. doi: 10.1093/jxb/erp096

Lee, T. D., Tjoelker, M. G., Reich, P. B., and Russelle, M. P. (2003). Contrasting growth response of an N_2 fixing and non fixing forb to elevated CO_2: dependence on soil N supply. *Plant Soil* 255, 475–486. doi: 10.1023/A:1026072130269

Li, Y. S., Liu, X. B., Wang, G. H., Yu, Z. H., Mathesius, U., Liu, J. D., et al. (2016). Shift in origin of plant nitrogen alters carbon and nitrogen assimilation during reproductive stages of soybean grown in a Mollisol. *Crop Pasture Sci.* 67, 872–880. doi: 10.1071/CP15184

Liu, N., Tian, Q., and Zhang, W. H. (2016). *Artemisia frigida* and *Stipa krylovii*, two dominant species in Inner Mongolia steppe, differed in their responses to elevated atmospheric CO_2 concentration. *Plant Soil* 409, 117–129. doi: 10.1007/s11104-016-2952-8

Liu, X. B., and Herbert, S. J. (2002). Fifteen years of research examining cultivation of continuous soybean in northeast China: a review. *Field Crops Res.* 79, 1–7. doi: 10.1016/S0378-4290(02)00042-4

Luo, Y. Q., Hui, D. F., and Zhang, D. Q. (2006). Elevated CO_2 stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. *Ecology* 87, 53–63. doi: 10.1890/04-1724

Makino, A., and Osmond, B. (1991). Effects of nitrogen nutrition on nitrogen partitioning between chloroplasts and mitochondria in pea and wheat. *Plant Physiol.* 96, 355–362. doi: 10.1104/pp.96.2.355

Mariotti, A., Lancelot, C., and Billen, G. (1984). Natural isotopic composition of nitrogen as a tracer of origin for suspended organic-matter in the scheldt estuary. *Geochim. Cosmochim. Acta* 48, 549–555. doi: 10.1016/0016-7037(84)90283-7

Martínez-Alcántara, B., Quiñones, A., Legaz, F., and Primo-Millo, E. (2012). Nitrogen-use efficiency of young citrus trees as influenced by the timing of fertilizer application. *J. Plant Nutr. Soil Sci.* 175, 282–292. doi: 10.1002/jpln.201100223

Matamala, R., and Schlesinger, W. H. (2000). Effects of elevated atmospheric CO_2 on fine root production and activity in an intact temperate forest ecosystem. *Global Change Biol.* 6, 967–979. doi: 10.1046/j.1365-2486.2000.00374.x

Matamala, R., Gonzalez-Meler, M. A., Jastrow, J. D., Norby, R. J., and Schlesinger, W. H. (2003). Impacts of fine root turnover on forest NPP and soil C sequestration potential. *Science* 302, 1385–1387. doi: 10.1126/science.1089543

Mikan, C. J., Zak, D. R., Kubiske, M. E., and Pregitzer, K. S. (2000). Combined effects of atmospheric CO_2 and N availability on the belowground carbon and nitrogen dynamics of aspen mesocosms. *Oecologia* 124, 432–445. doi: 10.1007/PL00008869

Miyagi, K. M., Kinugasa, T., Hikosaka, K., and Hirose, T. (2007). Elevated CO_2 concentration, nitrogen use, and seed production in annual plants. *Global Change Biol.* 13, 2161–2170. doi: 10.1111/j.1365-2486.2007.01429.x

Peng, S. B., Huang, J. L., Sheehy, J. E., Laza, R. C., Visperas, R. M., Zhong, X. H., et al. (2004). Rice yields decline with higher night temperature from global warming. *Proc. Natl. Acad. Sci. U.S.A.* 101, 9971–9975. doi: 10.1073/pnas.0403720101

Rennie, R. J., and Dubetz, S. (1986). N-15-determined nitrogen-fixation in field-grown chickpea, lentil, fababean, and field pea. *Agron. J.* 78, 654–660. doi: 10.2134/agronj1986.00021962007800040020x

Rogers, A., Ainsworth, E. A., and Leakey, A. D. B. (2009). Will elevated carbon dioxide concentration amplify the benefits of nitrogen fixation in legumes? *Plant Physiol.* 151, 1009–1016. doi: 10.1104/pp.109.144113

Rogers, A., Gibon, Y., Stitt, M., Morgan, P. B., Bernacchi, C. J., Ort, D. R., et al. (2006). Increased C availability at elevated carbon dioxide concentration improves N assimilation in a legume. *Plant Cell Environ.* 29, 1651–1658. doi: 10.1111/j.1365-3040.2006.01549.x

Rogers, H. H., Peterson, C. M., McCrimmon, J. N., and Cure, J. D. (1992). Response of plant-roots to elevated atmospheric carbon-dioxide. *Plant Cell Environ.* 15, 749–752. doi: 10.1111/j.1365-3040.1992.tb01018.x

Saeki, Y., Minami, M., Yamamoto, A., and Akao, S. (2008). Estimation of the bacterial community diversity of soybean-nodulating bradyrhizobia isolated from Rj-genotype soybeans. *Soil Sci. Plant Nutr.* 54, 718–724. doi: 10.1111/j.1747-0765.2008.00300.x

Salon, C., Munier-Jolain, N. G., Duc, G., Voisin, A. S., Grandgirard, D., Larmure, A., et al. (2001). Grain legume seed filling in relation to nitrogen acquisition: a review and prospects with particular reference to pea. *Agronomie* 21, 539–552. doi: 10.1051/agro:2001143

Sardans, J., and Peñuelas, J. (2012). The role of plants in the effects of global change on nutrient availability and stoichiometry in the plant-soil system. *Plant Physiol.* 160, 1741–1761. doi: 10.1104/pp.112.208785

Schiltz, S., Munier-Jolain, N., Jeudy, C., Burstin, J., and Salon, C. (2005). Dynamics of exogenous nitrogen partitioning and nitrogen remobilization from vegetative organs in pea revealed by ^{15}N *in vivo* labeling throughout seed filling. *Plant Physiol.* 137, 1463–1473. doi: 10.1104/pp.104.056713

Schneider, M. K., Luscher, A., Richter, M., Aeschlimann, U., Hartwig, U. A., Blum, H., et al. (2004). Ten years of free-air CO_2 enrichment altered the mobilization of N from soil in *Lolium perenne* L. swards. *Global Change Biol.* 10, 1377–1388. doi: 10.1111/j.1365-2486.2004.00803.x

Shimono, H., and Bunce, J. A. (2009). Acclimation of nitrogen uptake capacity of rice to elevated atmospheric CO_2 concentration. *Ann. Bot.* 103, 87–94. doi: 10.1093/aob/mcn209

Unkovich, M., and Baldock, J. (2008). Measurement of symbiotic N_2 fixation in Australian agriculture. *Soil Biol. Biochem.* 40, 2915–2921. doi: 10.1016/j.soilbio.2008.08.021

Warembourg, F. R., and Fernandez, M. P. (1985). Distribution and remobilization of symbiotically fixed nitrogen in soybean (*Glycine max*). *Physiol. Plant.* 65, 281–286. doi: 10.1111/j.1399-3054.1985.tb02396.x

Yu, Z. H., Li, Y. S., Wang, G. H., Liu, J. J., Liu, J. D., Liu, X. B., et al. (2016). Effectiveness of elevated CO_2 mediating bacterial communities in the soybean rhizosphere depends on genotypes. *Agr. Ecosyst. Environ.* 231, 229–232. doi: 10.1016/j.agee.2016.06.043

Zak, D. R., Pregitzer, K. S., Curtis, P. S., and Holmes, W. E. (2000). Atmospheric CO_2 and the composition and function of soil microbial communities. *Ecol. Appl.* 10, 47–59. doi: 10.1890/1051-0761(2000)010[0047:ACATCA]2.0.CO;2

Zanetti, S., Hartwig, U. A., Luscher, A., Hebeisen, T., Frehner, M., Fischer, B. U., et al. (1996). Stimulation of symbiotic N_2 fixation in *Trifolium repens* L. under elevated atmospheric pCO_2 in a grassland ecosystem. *Plant Physiol.* 112, 575–583. doi: 10.1104/pp.112.2.575

Zanetti, S., Hartwig, U. A., vanKessel, C., Luscher, A., Hebeisen, T., Frehner, M., et al. (1997). Does nitrogen nutrition restrict the CO_2 response of fertile grassland lacking legumes? *Oecologia* 112, 17–25.

Zhang, W. W., Wang, G. G., Liu, X. B., and Feng, Z. Z. (2014). Effects of elevated O_3 exposure on seed yield, N concentration and photosynthesis of nine soybean cultivars (*Glycine max* (L.) Merr.) in Northeast China. *Plant Sci.* 226, 172–181. doi: 10.1016/j.plantsci.2014.04.020

Ziska, L. H. (2008). Rising atmospheric carbon dioxide and plant biology: the overlooked paradigm. *DNA Cell Biol.* 27, 165–172. doi: 10.1089/dna.2007.0726

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Plant Science

ORIGINAL RESEARCH
published: 09 April 2018
doi: 10.3389/fpls.2018.00440

Bioethanol Potential of Energy Sorghum Grown on Marginal and Arable Lands

Chaochen Tang [1,2], Songbo Li [1,2], Meng Li [1,2] and Guang H. Xie [1,2]*

[1] College of Agronomy and Biotechnology, China Agricultural University, Beijing, China, [2] National Energy R&D Center for Non-food Biomass, China Agricultural University, Beijing, China

Field experiments were conducted in marginal lands, i.e., sub-humid climate and saline-land (SHS) and semi-arid climate and wasteland (SAW), to evaluate ethanol potential based on the biomass yield and chemical composition of biomass type (var. GN-2, GN-4, and GN-10) and sweet type (var. GT-3 and GT-7) hybrids of energy sorghum [*Sorghum bicolor* (L.) Moench] in comparison with sub-humid climate and cropland (SHC) in northern China. Results showed that environment significantly ($p < 0.05$) influenced plant growth, biomass yield and components, and subsequently the ethanol potential of energy sorghum. Biomass and theoretical ethanol yield of the crop grown at SHS (12.2 t ha^{-1} and 3,425 L ha^{-1}, respectively) and SAW (8.6 t ha^{-1} and 2,091 L ha^{-1}, respectively) were both statistically ($p < 0.001$) lower than values at the SHC site (32.6 t ha^{-1} and 11,853 L ha^{-1}, respectively). Higher desirable contents of soluble sugar, cellulose, and hemicellulose were observed at SHS and SHC sites, while sorghum grown at SAW possessed higher lignin and ash contents. Biomass type sorghum was superior to sweet type as non-food ethanol feedstock. In particular, biomass type hybrid GN-10 achieved the highest biomass (17.4 t ha^{-1}) and theoretical ethanol yields (5,423 L ha^{-1}) after averaging data for all environmental sites. The most productive hybrid, biomass type GN-4, exhibited biomass and theoretical ethanol yields >42.1 t ha^{-1} and 14,913 L ha^{-1}, respectively, at the cropland SHC site. In conclusion, energy sorghum grown on marginal lands showed a very lower ethanol potential, indicating a considerable lower possibility for being used as commercial feedstock supply when compared with that grown on regular croplands. Moreover, screening suitable varieties may improve energy sorghum growth and chemical properties for ethanol production on marginal lands.

Keywords: saline-land, dry wasteland, biomass sorghum, sweet sorghum, theoretical ethanol yield

Edited by:
José Manuel Mirás-Avalos, Universidade de Santiago de Compostela, Spain

Reviewed by:
Ilias Travlos, Agricultural University of Athens, Greece
Abdul-Sattar Nizami, King Abdulaziz University, Saudi Arabia

***Correspondence:**
Guang H. Xie
xiegh@cau.edu.cn

Specialty section:
This article was submitted to Agroecology and Land Use Systems, a section of the journal Frontiers in Plant Science

Received: *01 July 2017*
Accepted: *21 March 2018*
Published: *09 April 2018*

Citation:
Tang C, Li S, Li M and Xie GH (2018) Bioethanol Potential of Energy Sorghum Grown on Marginal and Arable Lands. Front. Plant Sci. 9:440. doi: 10.3389/fpls.2018.00440

INTRODUCTION

Industrial-scale cultivation of non-food energy crops for biofuels production is generally recognized as a positive step toward preventing energy shortages and decreasing greenhouse gas emissions (Qin et al., 2011; Sanscartier et al., 2014). As part of China's comprehensive energy plan, its bioenergy industry is vigorously accelerating cellulosic ethanol fuel production and diversifying feedstock supplies to include new crops such as cassava and sweet sorghum. In 2020, ethanol yield will reach 4.0 million tons, a 90% increase from 2.1 million tons in 2015, according to *the 13th 5-Year Plan for bioenergy development* released by the National Energy Administration of China.

Due to China's fairly limited cultivatable land resources, national policy has implemented land-use planning. As part of the overall plan, biofuel feedstock production will be limited to marginal lands to avoid land-use competition with food crops to maintain greater food security (Zhuang et al., 2011).

Energy sorghum, including biomass and sweet type varieties, has recently gained favor as bioethanol feedstock amongst numerous candidate crops (Rooney et al., 2007; Tew et al., 2008; Xie, 2012). Low input requirements, wide adaptability, and remarkable biological productivity confer better energy balance to sorghum as compared to other competing crops (Yu et al., 2008). Using current renewable energy technologies, soluble sugars and structural carbon compounds (cellulose and hemicellulose) in energy sorghum stems and leaves could be the most promising approach for the first and second generation ethanol production (Zhao et al., 2009; Zegada-Lizarazu and Monti, 2012; Cotton et al., 2013). Thus, knowledge of energy sorghum biomass chemical composition is a prerequisite for effective industrial production because composition directly impacts performance in various energy conversion processes. For example, cellulosic biomass is optimally converted to ethanol when lignin content is low (Weng et al., 2008). Lignin cannot be converted into carbohydrates and exerts a recalcitrant effect on conversion (Rocateli et al., 2012). In addition, high ash content may reduce efficiency of thermochemical conversion of biomass to fuel (Cassida et al., 2005).

The impact of environment factors including land type should be considered to select biomass feedstock crops and varieties. Rocateli et al. (2012) evaluated three types of sorghum (grain, forage, and photoperiod-sensitive sorghum) grown in the southern U.S. and observed that environment and genotype both exerted sizeable effects on biomass yield and chemical composition. Performances of biomass yield and its components of energy sorghum have been well documented by previous reports on the basis of its production on arable land (Amaducci et al., 2004; Tew et al., 2008; Zhao et al., 2009, 2012; Maw et al., 2016; Pannacci and Bartolini, 2016).

However, sorghum is particularly well-adapted to marginal land and constraints conditions, such as water deficits, salinity, and alkalinity (Dalla Marta et al., 2014; Regassa and Wortmann, 2014; Schmer et al., 2014). Sweet sorghum provided sufficient total sugar and ethanol yields in fields with a saline soil, even if it received 50–75% of the irrigation water typically applied to sorghum in Northern Greece (Vasilakoglou et al., 2011). On dryland in Nebraska one sweet sorghum cultivar was found to be competitive with grain crops for some biofuel criteria, but it was not competitive with grain crops for total or net liquid transportation fuel produced per hectare (Wortmann et al., 2010). Sweet sorghum exhibited a better energy efficiency (Ren et al., 2012) and economic return (Liu et al., 2015) to scale on investment than cotton or sunflower did on saline-alkali land in northern China. According to an industrial survey, the non-food feedstock cost was found to be 70–80% of the total ethanol production cost (Xie, 2012). Crop production in marginal lands faces a lack of infrastructural conditions and lower soil fertility, resulting in a higher feedstock cost than the same crop grown in regular croplands. However, previous reports comparing biomass yield and chemical composition of energy sorghum grown in marginal and croplands do not exist. Moreover, previous studies focused on sweet sorghum and few data are available on biomass sorghum, which has been recognized as a promising feedstock type for cellulosic ethanol production.

Therefore, the objectives of this study were: (1) to compare the variation in calculated ethanol potential based on biomass yield and chemical composition of energy sorghum grown on marginal and arable cropland under different climatic conditions; (2) to clarify the difference in biomass yield and chemical composition between biomass and sweet sorghum; and (3) to screen for suitable energy sorghum hybrids which could achieve high biomass yield and quality under marginal and arable land conditions for maximal ethanol production in northern China. The expected findings of this work could be helpful to evaluate the possibility of growing energy sorghum on marginal lands for commercial ethanol production in northern China.

MATERIALS AND METHODS

Site Description

Field experiments were conducted in northern China at three different sites with distinct environmental characteristics, i.e., sub-humid climate and saline-land (SHS), semi-arid climate and wasteland (SAW), and sub-humid climate and cropland (SHC) (**Table 1**). These locations were selected based on the results of Zhang et al. (2010), who reported that Inner Mongolia ranks the highest for ethanol production potential from sweet sorghum, followed by Hebei and next by the northern Shandong Province. Thus, these regions should be regarded as priority regions for energy sorghum based biofuel feedstock production in northern China. Soil samples at a depth of 0–30 cm were collected before sowing in order to determine the main soil physical and chemical properties (**Table 2**). Weather data for the three sites during the energy sorghum growth period were also collected from nearby meteorological stations.

Experimental Design and Operation

Five energy sorghum hybrids including biomass type (var. GN-2, GN-4, and GN-10) and sweet type (var. GT-3 and GT-7) were arranged in a randomized complete block design with four replicates at the SHS site in 2013, at the SWA site in 2013 and 2014, and at the SHC site in 2014. The selected hybrids were developed by the National Energy R&D Center for Non-food Biomass, China Agricultural University. Each plot was 36 m^2 in size and divided into a sampling area (12 m^2) and a harvest area (24 m^2) for all replicates. Because soil and meteorological conditions were different each year at each experimental site, thus each year-location combination was considered an "environment" with its own specific characteristics.

Two to three seeds were sown at 0.6 × 0.2 m intervals oriented in a north–south direction using a manual hill-drop method. At the three-leaf growth stage, seedlings were manually thinned to leave one vigorous plant per hole and concurrently weeds were manually removed. All trials were carried out in accordance with good agricultural practices. However, due to concerns about extreme soil and arid conditions at the SAW site, irrigation

TABLE 1 | Description of the study sites of sub-humid climate and saline-land (SHS), semi-arid climate and wasteland (SAW), and sub-humid climate and cropland (SHC).

Parameter	SHS site	SAW site	SHC site
Location	Binzhou, Shandong	Ordos, Inner Mongolia	Zhuozhou, Hebei
Latitude	37°42′N	39°10′N	39°28′N
Longitude	118°17′E	109°53′E	115°51′E
Elevation (m)	8	1,032	42
Climate type	Sub-humid	Semi-arid	Sub-humid
Land type	Saline-land	Wasteland	Cropland
Multi-year mean yearly precipitation (mm)	563	368	576
Multi-year mean yearly potential evaporation (mm)	1,213	2,506	1,575
Multi-year mean yearly average temperature (°C)	13	7	12
Multi-year mean yearly maximum temperature (°C)	19	12	19
Multi-year mean yearly minimum temperature (°C)	8	2	9

TABLE 2 | Main soil properties and meteorological characteristics during the growth period of energy sorghum at the experimental sites of sub-humid climate and saline-land (SHS), semi-arid climate and wasteland (SAW), and sub-humid climate and cropland (SHC).

Parameter	SHS site	SAW site		SHC site
	2013	2013	2014	2014
Sand[a] (%)	66.3	91.8	86.1	67.1
Silt[a] (%)	31.0	7.6	13.1	32.1
Clay[a] (%)	2.7	0.6	0.8	0.8
pH	8.2	8.0	8.1	7.9
Total salinity (g kg^{-1})	7.8	2.1	2.0	0.6
Soil organic matter (g kg^{-1})	5.4	0.7	0.7	12.0
Total nitrogen (g kg^{-1})	0.4	0.5	0.6	0.7
Available phosphorus (mg kg^{-1})	4.6	6.1	6.2	21.6
Available potassium (mg kg^{-1})	194.6	54.1	55.3	75.4
Rainfall (mm)	649.0	429.5	375.6	390.4
Relative humidity (%)	70.1	50.9	51.9	68.3
Daily mean temperature (°C)	24.4	18.8	18.9	23.0
Sunshine hours (h)	1044.0	1309.1	1163.4	1008.5
Solar radiation (MJ m^{-2})	2729.7	3029.5	3450.8	3013.2
Accumulated temperature (≥13°C)	1717.0	863.5	799.2	1704.3
Diurnal temperature difference (°C)	9.7	9.9	9.7	11.3

[a] *The soil texture was defined as sand, 0.02–2.0 mm; silt, 0.002–0.02 mm, and clay, <0.002 mm.*

and a higher fertilization dose were applied to the crop grown there, but not at the SHS and SHC sites. Sprinkler irrigation of approximately 30 mm of water was applied per month. Main agronomic practices and growth periods are presented in **Table 3**. The crop was harvested manually and harvest dates were chosen according to the timing of the killing frost.

Sample Collection and Measurements

On the harvest dates, tiller number was recorded for 10 hills in each plot and afterwards all aboveground plants in the harvest area of each plot were cut and weighed to estimate the fresh yield. Concurrently, 10 aboveground sorghum plants chosen randomly were harvested at the soil surface in the sampling area of each plot and were used to measure plant size (plant height and stem diameter). Next, each individual sample plant was divided into stems, leaves, and panicles, and their fresh weights were separately measured. For sampled stems, every other internode was taken from the base of each individual plant. All leaves, panicles, and sampled internodes were cut into pieces 2-to-3 cm in length and subsampled using a point-centered quarter method. Each subsample was weighed and oven-dried at 75°C until constant weight was achieved for gravimetric determination of moisture content and calculation of plant dry biomass yield.

Dried stem and leaf tissues (after panicles were removed) were ground using a Wiley mill and passed through a 0.5-mm mesh for total soluble sugar determination and through a 1-mm mesh for cellulose, hemicellulose, lignin, and ash determinations. Soluble sugar was determined in the supernatants using the anthrone-H_2SO_4 method and assayed using a UV–VIS spectrometer (TU-1901, Beijing Purkinje Instruments Co., Ltd., Beijing, China) according to Li et al. (2014). According to *National Renewable Energy Laboratory Analytical Procedures* (NREL LAP), cellulose, hemicellulose, and lignin were extracted using a two-step sulphuric acid hydrolysis process (Sluiter et al., 2008). Dry matter (2 g of each) was added to a 30 mL ceramic crucible to determine ash content using a muffle furnace (VULCAN 3-550, Dentsply International Inc., York, PA, USA). All chemical assays were conducted in triplicate and the average values were presented on an oven-dried basis.

Calculations and Statistical Analysis

Theoretical ethanol yield (TEY) values from soluble sugar, cellulose and hemicellulose were individually calculated using the following formulas:

$$\begin{aligned} TEY_{sugar} &= total\ sugar\ content \times dry\ biomass \times F1 \\ &\quad \times F2 \times \frac{1000}{\rho} \\ TEY_{cellu} &= cellulose\ and\ hemicellulose\ content \\ &\quad \times dry\ biomass \times F1 \times F2 \times F3 \times F4 \times \frac{1000}{\rho} \end{aligned}$$

Where, TEY_{sugar} represents the TEY from soluble sugar; TEY_{cellu} represents the TEY from cellulose and hemicellulose; F_1 represents the coefficient of conversion factor of ethanol from sugar (0.51); F_2 represents the process efficiency of ethanol from sugar (0.85); F_3 represents the coefficient of 1.11 for the conversion factor of sugar from cellulose and hemicellulose; F_4 represents the process efficiency of sugar from cellulose and hemicellulose (0.85); ρ represents the specific gravity of ethanol, 0.79 g mL^{-1}.

TABLE 3 | Agronomic practices in planting energy sorghum at the field experimental sites of sub-humid climate and saline-land (SHS), semi-arid climate and wasteland (SAW), and sub-humid climate and cropland (SHC).

Agronomic practice	SHS site	SAW site		SHC site
	2013	2013	2014	2014
Previous crop	Non	Non	Sorghum	Corn
Sowing date	1 May	8 May	12 May	29 April
Nitrogen fertilizer (kg N ha^{-1})	140	180	180	140
Phosphate fertilizer (kg P_2O_5 ha^{-1})	60	75	75	60
Potassium fertilizer (kg K_2O ha^{-1})	60	30	30	60
Irrigation (mm)	0	150	150	0
Harvest date	28 September	3 October	24 September	15 October
Growth duration (day)	151	149	136	170

Means and standard errors were calculated for the four replicates for each parameter. Two-way ANOVA was performed using the SPSS 19.0 analytical software package (IBM SPSS Inc., Chicago, IL, USA) to assess the effects of genotype, environment, and their interaction. A mean separation test was performed by using the *F*-protected least significant difference (LSD) test at 5% level of significance for each evaluated parameter. The coefficients of variation (CV) were calculated from all original determinations and defined as the ratio of the standard deviation to the mean value.

RESULTS AND DISCUSSION

Environmental Conditions

Soil and weather variables differed considerably during the energy sorghum growing period among the three sites (**Table 2**). Cumulative rainfall plus irrigation was higher at the SHS (649 mm) site and SWA (580 mm in 2013 and 526 mm in 2014) site than the SHC site (390 mm) during the sorghum growing seasons (**Tables 2**, **3**). Relative humidity, daily mean temperatures, and accumulated temperatures ($\geq 13°C$) were higher at the SHS and SHC sub-humid climate sites than the SAW semi-arid climate site, whereas cumulative sunshine hours and solar radiation varied inversely (**Table 2**). A maximum mean diurnal temperature difference value was observed at the SHC site, while the other sites exhibited almost no difference. Overall, the SHC site exhibited higher initial soil nutrients as compared to the marginal lands of both SHS and SAW sites.

Effect of Genotype and Environment on the Growth and Yield of Energy Sorghum

Effects of variables of environment, genotype, and their interaction on all measured parameters of plant growth were significant ($p < 0.05$), with the exception of non-significant effects of genotype on tiller number and ash yield and non-significant effects of environment and genotype interaction on tiller number, stem diameter, plant moisture, lignin content, and ash yield (**Table 4**). The effects of the studied factors on energy sorghum growth can be ranked as environment > genotype > interaction between genotype and environment. However, an exception to this ranking was observed in only one case, for soluble sugar content and hemicellulose content, where ranking was in the order of genotype > environment > environment and genotype interaction. These findings align with those of Amaducci et al. (2004), demonstrating that year, as well as the year and genotype interaction, had significant effects on aboveground biomass yield and quality of sweet and biomass sorghum. Furthermore, Zhao et al. (2009) concluded that effects of year and genotype on biomass, carbohydrates, and ethanol yield were highly significant ($p < 0.001$) and that differences among various years were ultimately attributed to variations in environmental conditions.

TABLE 4 | Combined analyses of variance (*F*-value) for morphological and chemical characteristics of energy sorghum evaluated for four environments under field conditions.

Parameter	Environment ($df = 3$)	Genotype ($df = 4$)	Environment × Genotype ($df = 12$)
Tiller number	15.6^{***}	2.3^{ns}	0.9^{ns}
Plant height	631.0^{***}	79.9^{***}	2.7^{**}
Stem diameter	24.9^{***}	9.7^{***}	1.4^{ns}
Plant moisture	139.8^{***}	6.7^{***}	0.9^{ns}
Biomass yield	231.3^{***}	4.9^{**}	3.2^{**}
Soluble sugar content	21.0^{***}	29.7^{***}	4.5^{***}
Soluble sugar yield	195.3^{***}	12.3^{***}	3.8^{***}
Cellulose content	54.2^{***}	28.4^{***}	3.6^{**}
Cellulose yield	291.0^{***}	11.1^{***}	5.8^{***}
Hemicellulose content	20.6^{***}	42.6^{***}	7.8^{***}
Hemicellulose yield	277.6^{***}	13.5^{***}	7.0^{***}
Lignin content	50.9^{***}	3.7^{*}	1.4^{ns}
Lignin yield	205.9^{***}	9.9^{***}	6.7^{***}
Ash content	85.3^{***}	10.5^{***}	2.8^{**}
Ash yield	105.7^{***}	1.5^{ns}	1.6^{ns}
Theoretical ethanol yield	301.3^{***}	4.7^{**}	2.8^{**}

^{ns}*Non-significant effects;*
**Significant effect at* $p < 0.05$ *level;*
***Significant effect at* $p < 0.01$ *level;*
****Significant effect at* $p < 0.001$ *level.*

Tiller Number, Plant Size, and Moisture Content

Tiller number, plant size, and moisture content showed significant differences ($p < 0.05$) among the experimental sites and the energy sorghum hybrids (**Tables 5**, **6**). Averaged across all the hybrids, both SAW, and SHS sites produced plants with smaller size, higher tiller number, and higher plant moisture content in comparison with plants of the SHC site (**Table 5**), whereas each of these parameters was lower for sorghum at the SAW site vs. the SHS site. Moreover, biomass type hybrids exhibited larger plant sizes than sweet type hybrids did, whereas

TABLE 5 | Energy sorghum characteristics for performance at the experimental sites of sub-humid climate and saline-land (SHS), semi-arid climate and wasteland (SAW), and sub-humid climate and cropland (SHC).

Character	SHS site	SAW site		SHC site
	2013	2013	2014	2014
Plant height (cm)	444 a	258 b	227 c	444 a
Stem diameter (mm)	17.3 a	15.1 b	14.9 c	17.0 a
Tiller number (no.)	0.5 a	0.2 b	0.2 b	0.2 b
Plant moisture (%)	83.6 a	66.6 c	72.9 b	65.3 c
Soluble sugar content (g kg^{-1})	171 b	111 c	118 c	201 a
Soluble sugar yield (t ha^{-1})	1.3 b	0.7 c	0.7 c	5.9 a
Cellulose content (g kg^{-1})	384 a	345 b	298 d	330 c
Cellulose yield (t ha^{-1})	3.2 b	2.0 c	1.8 c	10.0 a
Hemicellulose content (g kg^{-1})	238 a	239 a	226 b	206 c
Hemicellulose yield (t ha^{-1})	2.0 b	1.4 c	1.4 c	6.3 a
Lignin content (g kg^{-1})	173 b	247 a	155 b	158 b
Lignin yield (t ha^{-1})	1.5 b	1.5 b	0.9 c	4.8 a
Ash content (g kg^{-1})	40 c	56 b	61 a	30 d
Ash yield (t ha^{-1})	0.3 b	0.3 b	0.3 b	0.9 a

Different small letters within a row indicate significant differences at $p < 0.05$.

TABLE 6 | Plant size, tiller number, and plant moisture of the energy sorghum hybrids averaged across the experimental sites of sub-humid climate and saline-land (SHS), semi-arid climate and wasteland (SAW), and sub-humid climate and cropland (SHC).

Type	Hybrid	Plant height (cm)	Stem diameter (mm)	Tiller number (no.)	Plant moisture (%)
Biomass sorghum	GN-2	363 b	16.2 ab	0.2 ab	71.7 b
	GN-4	363 b	17.0 a	0.2 b	71.1 b
	GN-10	379 a	16.2 ab	0.3 a	68.7 c
	Average	368	16.5	0.2	70.5
Sweet sorghum	GT-3	312 c	15.8 b	0.3 a	74.7 a
	GT-7	262 d	14.7 c	0.3 ab	71.3 b
	Average	287	15.2	0.3	73.0

Regardless of sorghum type, different small letters within a column indicate significant differences at $p < 0.05$.

tiller number and plant moisture were higher in sweet type hybrids (**Table 6**).

In general, larger plant size is partially responsible for the highest observed biomass yield at the SHC site and showed a significantly positive correlation ($p < 0.01$) with biomass yield ($r = 0.663$ for plant height and $r = 0.471$ for stem diameter). In addition, the longer growth period at the SHC site also contributed to higher biomass yield, as did lower tiller number, as observed previously (Huang et al., 2013). Moreover, Ao et al. (2010) demonstrated that low tiller number values can facilitate synchronous harvest by promoting uniformity of plant characteristics, ensuring a more efficient use of horizontal space. Furthermore, low plant moisture of biomass sorghum is very conducive to rapid drying for facilitated transportation and storage (Zegada-Lizarazu and Monti, 2012; Iqbal et al., 2017).

Biomass Yield and Stem, Leaf, Panicle Partitioning

Obviously, biomass yields averaged across all energy sorghum hybrids grown at either the SAW site (8.6 t ha^{-1} for average of 2013 and 2014) or SHS site (12.2 t ha^{-1}) were statistically ($p < 0.01$) lower compared to average yield for hybrids grown at the SHC site (32.6 t ha^{-1}) (**Figure 1**). However, energy sorghum at the SHS site showed a significantly ($p < 0.05$) higher biomass yield (41.9%) than at the SAW site. In general, salt stress at the SHS site or infertile soil coupled with higher evaporation probably leading to soil water stress at the SAW site decrease biomass yield relative to the regular cropland conditions at the SHC site. The dramatic differences in biomass yield at different sites in this study could be attributed to considerable diversity in environmental factors, such as climate (precipitation, temperature, and evaporation) and soil type and fertility. Tang et al. (2015) demonstrated that precipitation and soil organic matter were key environmental factors influencing biomass yield of sweet sorghum. Meanwhile, high altitude also caused a decline in sweet sorghum production due to a lower temperature (Li and Feng, 2013). Previous studies confirmed that well-timed irrigation could considerably improve biomass yield (Mastrorilli et al., 1995; Dercas and Liakatas, 2007). Habyarimana et al. (2004) demonstrated that higher aboveground biomass yield of sorghum ranged from 33 to 51 t ha^{-1} under irrigation than that of 20–29 t ha^{-1} under rain-fed conditions in the Mediterranean region. Cosentino et al. (2012) reported that sweet sorghum produced 7.5 t ha^{-1} of dry matter with 80 mm irrigation vs. 21.1 t ha^{-1} with 334 mm irrigation under semi-arid conditions.

While lower than cropland biomass yields, yields on marginal lands studied here were comparable to yields of previous field studies conducted under similar environmental conditions. For instance, Ameen et al. (2017) and Fu et al. (2016) measured biomass yield of energy sorghum fluctuating from 4.9 to 14.2 t ha^{-1} on a sandy loam soil of marginal land in Inner Mongolia. A recent study by Tang et al. (2018) reported that energy sorghum exhibited a good biomass yield (6.1–9.2 t ha^{-1}) due to its superior adaptability to abandoned marginal land. In another study conducted in northern Greece, significantly lower sweet sorghum biomass yield (13.7 t ha^{-1}) was observed in soil with high salinity (Vasilakoglou et al., 2011).

Averaged across hybrids, biomass type sorghum exhibited significantly ($p < 0.05$) higher biomass yield (17.3 t ha^{-1}) than sweet type (14.7 t ha^{-1}), with a particularly greater difference in biomass type vs. sweet type yields at the SHC site (34.5 vs. 29.9 t ha^{-1}, respectively) (**Figure 1**). Thus, biomass type sorghum holds a promising future for energy generation due to its higher biomass production compared to that of sweet type sorghum in this study. With regard to two type's hybrids across all sites, biomass type hybrid GN-10 showed the highest average biomass yield (17.4 t ha^{-1}) and is particularly well-adapted to adverse environmental conditions such as water deficits, salinity, and alkalinity. Considering only biomass yield performance as the major priority, biomass type hybrid GN-4 demonstrated a very high biomass yield of 42.1 t ha^{-1} after growth on cropland (but not on marginal land) under sub-humid climate conditions at the SHC site. Other research groups have also achieved successful growth of energy sorghum in sub-humid climate conditions, including Gnansounou et al. (2005) who reported that sorghum for energy purpose was well adapted to temperate sub-humid climates, and Zhao et al. (2009), who reported that sweet sorghum exhibited a high biomass yield of 35.2 t ha^{-1} after 40 days following anthesis under sub-humid climate conditions.

Biomass yield partitioning across all the hybrids showed that stem weight represented the highest proportion (74.8–82.3%) of total dry biomass at the SHC site to the values at the SHS site (50.4–66.1%) and SAW site (39.5–60.2%). Panicle biomass was found to be significantly ($p < 0.05$) the lowest proportion of total biomass, ranging between 4.6 and 9.7% at SHC site (**Figure 2**).

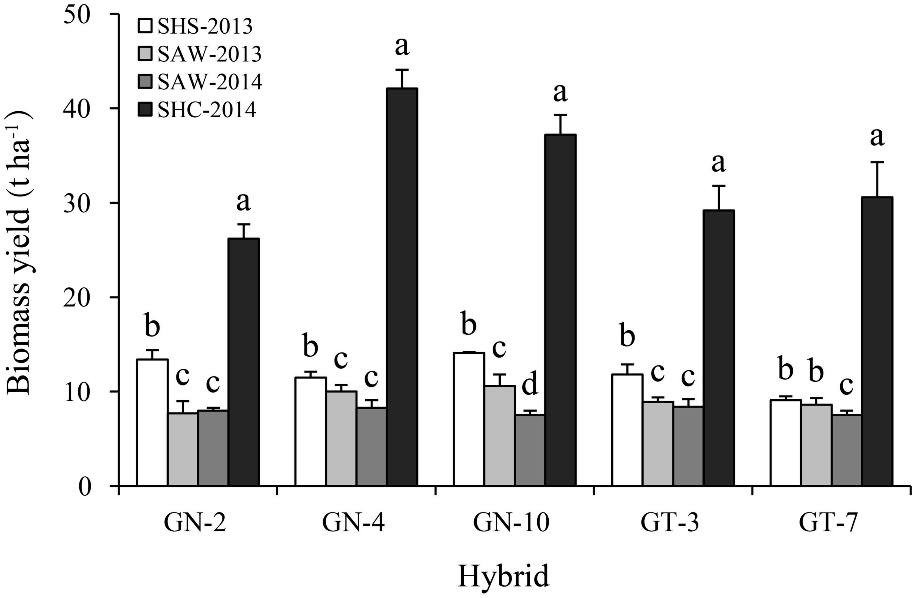

FIGURE 1 | Biomass yield of energy sorghum hybrids at sites of sub-humid climate and saline-land (SHS), semi-arid climate and wasteland (SAW), and sub-humid climate and cropland (SHC) in 2013 and 2014. The different small letters indicate significant differences within environments for each hybrid at the $p < 0.05$ level. Vertical bars represent standard errors.

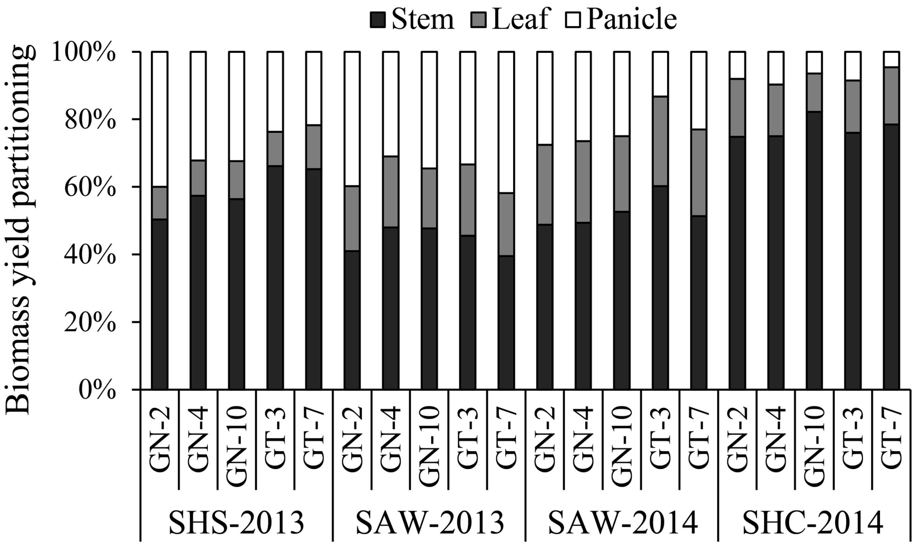

FIGURE 2 | Dry biomass yield partitioning of energy sorghum hybrids as determined by the weight fractions of stem, leaf, and panicle for different experimental sites of sub-humid climate and saline-land (SHS), semi-arid climate and wasteland (SAW), and sub-humid climate and cropland (SHC) in 2013 and 2014.

Notably, sweet type sorghum hybrids exhibited higher overall values of stem (60.3 vs. 57.0%) and leaf biomass yield (18.4 vs. 16.9%) than biomass type hybrids.

Chemical Components

Energy sorghum chemical components were significantly affected by environment and sorghum genotype. Across all sites, a relatively high coefficient of variation (CV) was observed for soluble sugar (34.5%), lignin (26.1%), and ash (33.7%), whereas cellulose and hemicellulose content exhibited relatively lower variability, with CV values of 13.4 and 10.4%, respectively (**Figure 3**). Previous studies reported that sucrose, cellulose, hemicellulose, and ash content varied significantly with locations, while lignin content remained relatively constant (Amaducci et al., 2004; Singh et al., 2012; Wei et al., 2014). After comparison of the three sites in this study (**Table 5**), we determined that under sub-humid climate conditions, the SHC site was most conducive to obtaining ideal soluble sugar content, while the SHS site was conducive to obtaining higher cellulose and hemicellulose content. However, higher content of lignin and ash observed for sorghum from the SAW site demonstrated that undesirable components of cellulosic materials may easily be produced on sandy wasteland under the water deficit conditions of a semi-arid region. Therefore, energy sorghum cultivated in a sub-humid climate is recommended instead for use as solid biofuel feedstock for thermal utilization, due to its lower ash content (Pannacci and Bartolini, 2016).

Meanwhile, yield of all chemical components in aboveground plants was significantly ($p < 0.05$) higher at the SHC site (**Table 5**), due to significantly higher overall biomass production.

TABLE 7 | Content and yield of chemical components in plants of different energy sorghum hybrids averaged across the experimental sites of sub-humid climate and saline-land (SHS), semi-arid climate and wasteland (SAW), and sub-humid climate and cropland (SHC).

Type	Hybrid	Soluble sugar	Cellulose	Hemicellulose	Lignin	Ash
Content	Biomass sorghum					
(g kg^{-1})	GN-2	101 b	359 a	246 a	196 a	49 ab
	GN-4	111 b	358 a	243 a	198 a	46 b
	GN-10	114 b	363 a	247 a	184 ab	39 c
	Average	109	360	245	193	42
	Sweet sorghum					
	GT-3	214 a	304 b	200 b	169 b	47 b
	GT-7	207 a	315 b	201 b	172 b	54 a
	Average	210	310	201	171	50
Yield	Biomass sorghum					
(t ha^{-1})	GN-2	1.3 b	3.9 a	2.6 a	2.0 b	0.4 b
	GN-4	1.8 b	4.9 a	3.2 a	2.6 a	0.5 ab
	GN-10	1.8 b	5.3 a	3.4 a	2.6 a	0.5 b
	Average	1.6	4.7	3.1	2.4	0.5
	Sweet sorghum					
	GT-3	3.1 a	3.6 b	2.2 b	1.8 b	0.5 ab
	GT-7	2.9 a	3.9 b	2.5 b	1.9 b	0.6 a
	Average	3.0	3.7	2.3	1.8	0.6

Regardless of sorghum type, different small letters within a column indicate significant differences at p < 0.05.

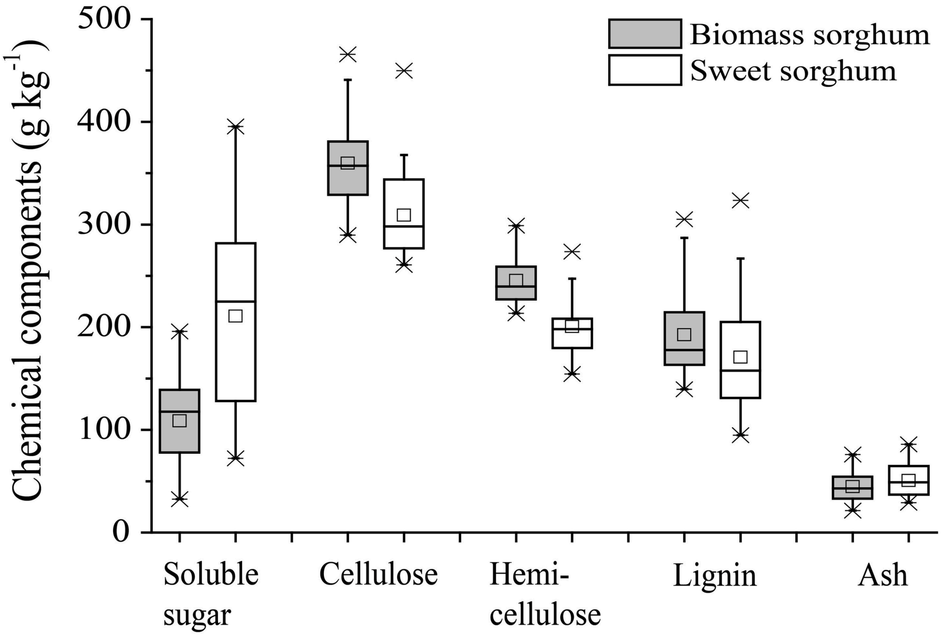

FIGURE 3 | Variations in chemical components of whole plants of biomass and sweet sorghum at the experimental sites of sub-humid climate and saline-land (SHS), semi-arid climate and wasteland (SAW), and sub-humid climate and cropland (SHC).

In particular, the yields of three desirable components (soluble sugar, cellulose, and hemicellulose) on marginal lands were 4.5–8.4 times lower at the SAW site (average of 2013 and 2014) than the SHC site and 3.2–4.5 times lower at the SHS site than at the SHC site. On the one hand, water supply and normal agricultural land for conservation tillage positively affected cellulosic biomass production (Rocateli et al., 2012). On the other hand, for energy purpose total cellulosic biomass yield is much more important than cellulosic biomass quality for selection of the optimal energy sorghum hybrids.

As an additional consideration, biomass type sorghum is predominantly composed of structural carbohydrates (cellulose and hemicellulose) (**Figure 3**). It exhibited significantly ($p < 0.01$) higher (by 27.0–34.8%) yields of cellulose,

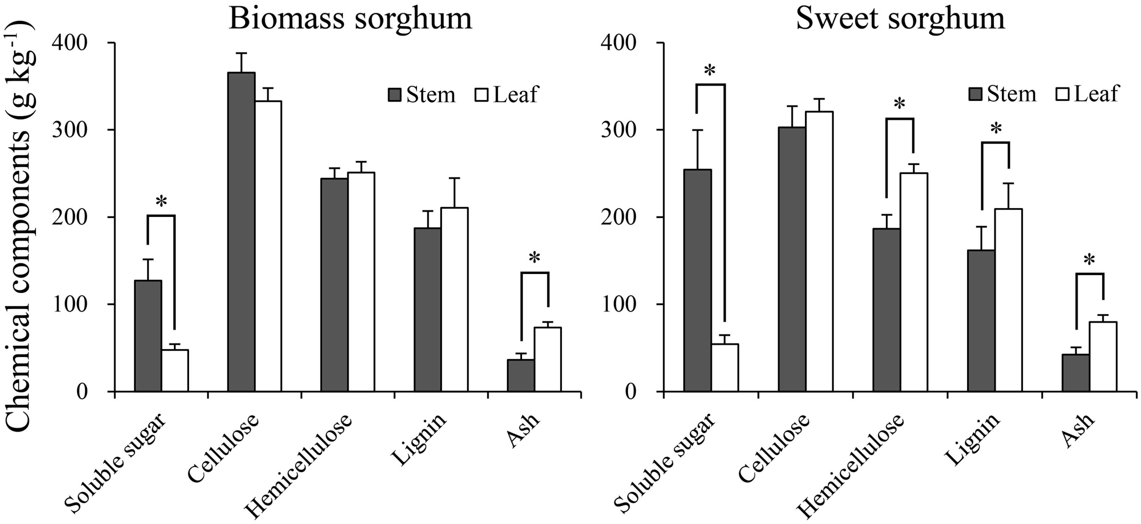

FIGURE 4 | Chemical components partitioning of biomass and sweet sorghum into stem and leaf averaged across all hybrids used at the experimental sites of sub-humid climate and saline-land (SHS), semi-arid climate and wasteland (SAW), and sub-humid climate and cropland (SHC). Asterisks indicate significant differences within stem and leaf for each component at the $p < 0.05$ level. Vertical bars represent standard errors.

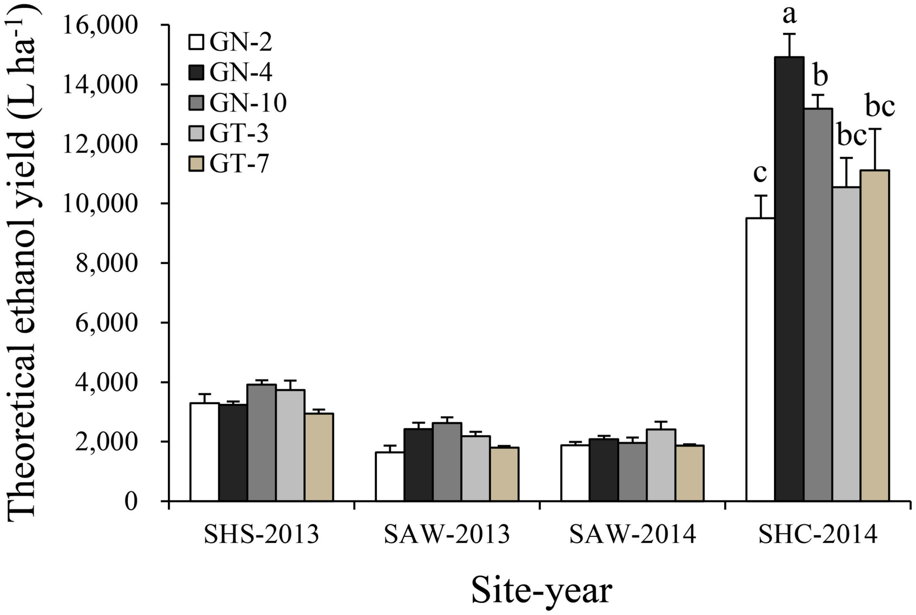

FIGURE 5 | Theoretical ethanol yield of energy sorghum hybrids at sites of sub-humid climate and saline-land (SHS), semi-arid climate and wasteland (SAW), and sub-humid climate and cropland (SHC) in 2013 and 2014. The different small letters indicate significant differences within each hybrid and each site at the $p < 0.05$ level. The vertical bars indicate standard errors.

hemicellulose, and lignin than the sweet type. However, reverse trends were observed for yields of soluble sugars and ash, which were lower (by 87.5 and 20%, respectively) for biomass type sorghum when averaged across all hybrids and sites (**Table 7**). Moreover, hybrid GN-10 biomass type sorghum exhibited higher contents of desirable components (including soluble sugar, cellulose, and hemicellulose) and lower contents of lignin and ash in aboveground plants, while producing the highest yields (10.5 t ha^{-1}) of the first three aforementioned components across all sites. Between the two hybrids of sweet type sorghum analyzed, GT-7 produced higher yields of all chemical components except for the yield of soluble sugar.

With regard to components partitioning, soluble sugar in stem was significantly ($p < 0.05$) higher (3.7 times) than in leaf when averaged across sites and hybrids (**Figure 4**). Moreover, ratios of components in leaf vs. stem were as follows: hemicellulose content (1.2 times), lignin (1.2 times), and ash (1.9 times). However, while cellulose content was 9.9% higher in stem than in leaf of biomass type sorghum, cellulose was 6.0% lower in stem than leaf of sweet type sorghum. These findings agreed with results of Zhao et al. (2009) and Monti et al. (2008).

Theoretical Ethanol Yield (TEY)

High TEY yield mirrored biomass yield in this study; a TEY >11,853 L ha^{-1} was observed at the SHC site, which produced 3.5 times ($p < 0.05$) higher ethanol yield than that observed at the SHS site (3,425 L ha^{-1}) and 5.7 times greater yield than at the SAW site (2,091 L ha^{-1}, averaged of 2013 and 2014) (**Figure 5**). Furthermore, correlation analysis of biomass yield, plant height, stem diameter, and soluble sugar content showed significantly ($p < 0.01$) positive correlations with TEY; however, the content of ash, lignin, and hemicellulose and plant moisture were negatively correlated with TEY ($p < 0.01$, **Figure 6**). However, tiller number and cellulose content were not significantly correlated with TEY, which indicates that both parameters did not affect ethanol production.

The TEY values for marginal lands including saline-land and dry wasteland reflected severely reduced potential ethanol production relative to cropland. According to Fu et al. (2016), sweet sorghum grown on sandy loam soil exhibited TEY of 2,491 L ha^{-1} from stalk of the crop in a semi-arid region in northern China. Vasilakoglou et al. (2011) reported an ethanol yield of 2,623 L ha^{-1} from sweet sorghum on land with salinity 6.9 dS m^{-1}. Wortmann et al. (2010) reported a potential ethanol yield of 2,211 L ha^{-1} using biomass of sweet sorghum grown at seven dryland site-years in a semi-arid region. However, much higher ethanol yield on cropland under sub-humid climate conditions at Missouri, USA, was reported by Houx and Fritschi (2013) and Maw et al. (2016), indicating that sweet sorghum can achieve TEY values of 5,000–7,488 L ha^{-1}. Moreover, Zhao et al. (2012) reported that high-yielding sweet sorghum cultivars provided the highest ethanol yield potential ranging between 9,097 and 10,803 L ha^{-1} from sugar, starch, cellulose, and hemicellulose, on a cropland geographically near to the SHC site of this study. The reason for the large gap of ethanol potential from sweet sorghum between marginal land and cropland would probably be the variations in temperature, precipitation, evaporation, soil fertility, and management practices, which could substantially impact crop biomass yield and components.

In this study, the biomass type sorghum exhibited a higher TEY magnitude compared with sweet type sorghum (5,056 vs. 4,578 L ha^{-1}) averaged across all sites. In particular, hybrid GN-10 biomass type sorghum produced the highest TEY (5,423 L ha^{-1}, **Figure 5**), which was 34.1% higher than the lowest TEY observed for hybrid GN-2. Hybrid GN-4 produced significantly ($p < 0.05$) highest ethanol yield at the SHC site relative to the other hybrids, exhibiting the highest value of 14,913 L ha^{-1}.

Future Perspectives

In this study, energy sorghum grown on marginal lands exhibited a much lower ethanol potential than that on cropland, indicating a considerable lower possibility for being used as commercial feedstock production due to environmental stresses and an additional input. At a saline-alkali site Wuyuan in northern China, sweet sorghum showed negative economic performance, whereas the reference crops maize and sunflower exhibited relatively high positive benefit (Liu et al., 2015). For sustainable commercial energy sorghum production, marginal lands with relatively low environmental stresses should be selected and stress-resistant plantation technologies should be developed. It is important to screen stress-resistant varieties with genetic improvement strategy and establish efficient crop production systems with conservation tillage (Xie, 2012). Favorable policy is particularly of significance in non-food biofuel development. Economic incentives including specific capital subsidies, low-cost financing, tax incentives and R&D funding should be

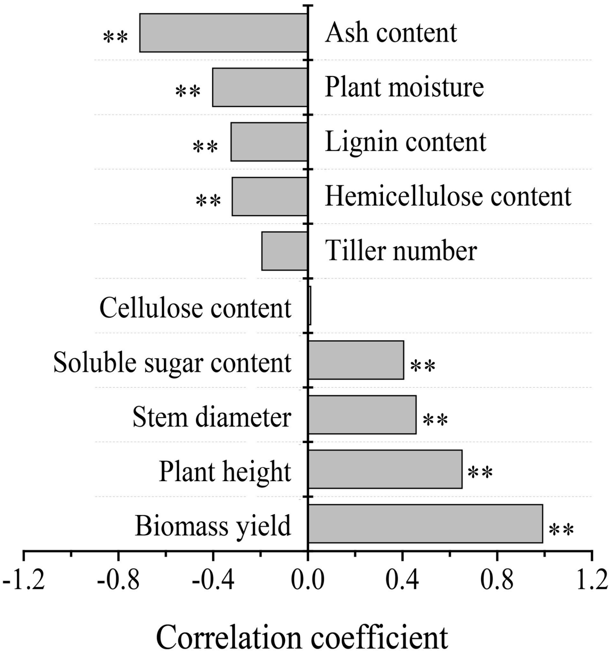

FIGURE 6 | Analysis of correlation between theoretical ethanol yield and related growth, yield and quality parameters of energy sorghum production. Two asterisks indicate significant correlations at the $p < 0.01$ level.

established to promote non-food energy crop production in marginal lands.

CONCLUSIONS

This study revealed environmental stress affecting biomass yield to guide future development of promising sorghum hybrids adapted to growth on marginal lands. As part of a larger sustainable agro-industrial framework, biomass type sorghum feedstock should be encouraged for industrial scale ethanol production due to its high productivity, adaptation to marginal growth conditions, and desirable qualities that facilitate efficient conversion of its biomass to ethanol. In particular, hybrid biomass type GN-10 possesses all of these attributes, while being especially well-adapted to growth in adverse environmental conditions such as water deficits, salinity, and alkalinity. However, from an output point of view, biomass type hybrid GN-4 achieved the highest values of biomass yield (42.1 t ha^{-1}) and TEY (14,913 L ha^{-1}) on cropland in a sub-humid climate. Ultimately, lower ethanol potential of energy sorghum grown on marginal land reflected a lower possibility for commercial feedstock supply than that grown on regular cropland. As well, screening suitable varieties could improve energy sorghum growth and chemical components for ethanol production.

AUTHOR CONTRIBUTIONS

CT: Analyzed the data, made tables and figures, and drafted the manuscript. SL and ML: Performed the laboratory experiments and collected the data. GX: Designed and supervised the field and laboratory work and finalized the manuscript.

FUNDING

This work was supported by the National Natural Science Foundation of China (31470555), China Datang New Energy Co. Ltd., and Henan Tianguan Group Co., Ltd.

ACKNOWLEDGMENTS

We would like to thank Mr. Asif Ameen for his downloading the literature and helping to improve language in this manuscript.

REFERENCES

Amaducci, S., Monti, A., and Venturi, G. (2004). Non-structural carbohydrates and fibre components in sweet and fibre sorghum as affected by low and normal input techniques. *Ind. Crop Prod*. 20, 111–118. doi: 10.1016/j.indcrop.2003.12.016

Ameen, A., Yang, X., Chen, F., Tang, C., Du, F., Fahad, S., et al. (2017). Biomass yield and nutrient uptake of energy sorghum in response to nitrogen fertilizer rate on marginal land in a semi-arid region. *Bioenerg. Res*. 10, 363–376. doi: 10.1007/s12155-016-9804-5

Ao, H., Peng, S., Zou, Y., Tang, Q., and Visperas, R. M. (2010). Reduction of unproductive tillers did not increase the grain yield of irrigated rice. *Field Crop Res*. 116, 108–115. doi: 10.1016/j.fcr.2009.11.020

Cassida, K. A., Muir, J. P., Hussey, M. A., Read, J. C., Venuto, B. C., and Ocumpaugh, W. R. (2005). Biofuel component concentrations and yields of switchgrass in South Central, U.S. *Environ. Crop Sci*. 45, 682–692. doi: 10.2135/cropsci2005.0682

Cosentino, S. L., Mantineo, M., and Testa, G. (2012). Water and nitrogen balance of sweet sorghum (*Sorghum bicolor* moench (L.)) cv. Keller under semi-arid conditions. *Ind. Crop Prod*. 36, 329–342. doi: 10.1016/j.indcrop.2011.10.028

Cotton, J., Burow, G., Acosta-Martinez, V., and Moore-Kucera, J. (2013). Biomass and cellulosic ethanol production of forage sorghum under limited water conditions. *Bioenerg. Res*. 6, 711–718. doi: 10.1007/s12155-012-9285-0

Dalla Marta, A., Mancini, M., Orlando, F., Natali, F., Capecchi, L., and Orlandini, S. (2014). Sweet sorghum for bioethanol production: crop responses to different water stress levels. *Biomass Bioenerg*. 64, 211–219. doi: 10.1016/j.biombioe.2014.03.033

Dercas, N., and Liakatas, A. (2007). Water and radiation effect on sweet sorghum productivity. *Water Resour. Manage*. 21, 1585–1600. doi: 10.1007/s11269-006-9115-2

Fu, H. M., Meng, F. Y., Molatudi, R. L., and Zhang, B. G. (2016). Sorghum and switchgrass as biofuel feedstocks on marginal lands in Northern China. *Bioenerg Res*. 9, 633–642. doi: 10.1007/s12155-015-9704-0

Gnansounou, E., Dauriat, A., and Wyman, C. E. (2005). Refining sweet sorghum to ethanol and sugar: economic trade-offs in the context of North China. *Bioresour. Technol*. 96, 985–1002. doi: 10.1016/j.biortech.2004.09.015

Habyarimana, E., Laureti, D., Ninno, M. D., and Lorenzoni, C. (2004). Performances of biomass sorghum [*Sorghum bicolor* (L.) Moench] under different water regimes in Mediterranean region. *Ind. Crop Prod*. 20, 23–28. doi: 10.1016/j.indcrop.2003.12.019

Houx, J. H., and Fritschi, F. B. (2013). Influence of midsummer planting dates on ethanol production potential of sweet sorghum. *Agron. J*. 105, 1761–1768. doi: 10.2134/agronj2013.0083

Huang, M., Yang, C., Ji, Q., Jiang, L., Tan, J., and Li, Y. (2013). Tillering responses of rice to plant density and nitrogen rate in a subtropical environment of southern China. *Field Crop Res*. 149, 187–192. doi: 10.1016/j.fcr.2013.04.029

Iqbal, T., Nizami, A. S., Eckhoff, S., Barreto, M. L. A., Sadef, Y., Rehan, M., et al. (2017). Biomass conservation using an optimized drying process for energy sorghum bagasse. *Renew. Energ. Focus* 19–20, 1–7. doi: 10.1016/j.ref.2017.03.005

Li, C. X., and Feng, H. S. (2013). A study on the adaptability of sweet sorghum planted in different altitudinal areas of the Qinghai plateau. *Acta Pratac. Sin*. 22, 51–59 (in Chinses with English abstract). doi: 10.11686/cyxb20130307

Li, M., Feng, S., Wu, L., Li, Y., Fan, C., Zhang, R., et al. (2014). Sugar-rich sweet sorghum is distinctively affected by wall polymer features for biomass digestibility and ethanol fermentation in bagasse. *Bioresour. Technol*. 167, 14–23. doi: 10.1016/j.biortech.2014.04.086

Liu, H., Ren, L., Spiertz, H., Zhu, Y., and Xie, G. H. (2015). An economic analysis of sweet sorghum cultivation for ethanol production in North China. *GCB Bioenerg*. 7, 1176–1184. doi: 10.1111/gcbb.12222

Mastrorilli, M., Katerji, N., Rana, G., and Steduto, P. (1995). Sweet sorghum in Mediterranean climate: radiation use and biomass water use efficiencies. *Ind. Crop Prod*. 3, 253–260. doi: 10.1016/0926-6690(94)00002-G

Maw, M. J. W., Houx, J. H., and Fritschi, F. B. (2016). Sweet sorghum ethanol yield component response to nitrogen fertilization. *Ind. Crop Prod*. 84, 43–49. doi: 10.1016/j.indcrop.2016.01.038

Monti, A., Di Virgilio, N., and Venturi, G. (2008). Mineral composition and ash content of six major energy crops. *Biomass Bioenerg*. 32, 216–223. doi: 10.1016/j.biombioe.2007.09.012

Pannacci, E., and Bartolini, S. (2016). Evaluation of sorghum hybrids for biomass production in central Italy. *Biomass Bioenerg*. 88, 135–141. doi: 10.1016/j.biombioe.2016.03.024

Qin, Z., Zhuang, Q., Zhu, X., Cai, X., Zhang, X. (2011). Carbon consequences and agricultural implications of growing biofuel crops on marginal agricultural lands in China. *Environ. Sci. Technol*. 45, 10765–10772. doi: 10.1021/es2024934

Regassa, T. H., and Wortmann, C. S. (2014). Sweet sorghum as a bioenergy crop: literature review. *Biomass Bioenerg*. 64, 348–355. doi: 10.1016/j.biombioe.2014.03.052

Ren, L. T., Liu, Z. X., Wei, T. Y., and Xie, G. H. (2012). Evaluation of energy input and output of sweet sorghum grown as a bioenergy crop on coastal saline-alkali land. *Energy* 47, 166–173. doi: 10.1016/j.energy.2012.09.024

Rocateli, A. C., Raper, R. L., Balkcom, K. S., Arriaga, F. J., and Bransby, D. I. (2012). Biomass sorghum production and components under different irrigation/tillage systems for the southeastern U.S. *Ind. Crop Prod*. 36, 589–598. doi: 10.1016/j.indcrop.2011.11.007

Rooney, W. L., Blumenthal, J., Bean, B., and Mullet, J. E. (2007). Designing sorghum as a dedicated bioenergy feedstock. *Biofuels Bioprod. Bioref.* 1, 147–157. doi: 10.1002/bbb.15

Sanscartier, D., Deen, B., Dias, G., Maclean, H., Dadfar, H., Mcdonald, I., et al. (2014). Implications of land class and environmental factors on life cycle GHG emissions of Miscanthus as a bioenergy feedstock. *GCB Bioenerg.* 6, 401–413. doi: 10.1111/gcbb.12062

Schmer, M. R., Vogel, K. P., Varvel, G. E., Follett, R. F., and Mitchell, R. B. (2014). Energy potential and greenhouse gas emissions from bioenergy cropping systems on marginally productive cropland. *PLoS ONE* 9:e89501. doi: 10.1371/journal.pone.0089501

Singh, M. P., Erickson, J. E., Sollenberger, L. E., Woodard, K. R., Vendramini, J. M. B., and Fedenko, J. R. (2012). Mineral composition and biomass partitioning of sweet sorghum grown for bioenergy in the southeastern USA. *Biomass Bioenerg*. 47, 1–8. doi: 10.1016/j.biombioe.2012.10.022

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., et al. (2008). *Determination of Structural Carbohydrates and Lignin in Biomass.* NREL/TP-510–42618. Colorado: National Renewable Energy Laboratory.

Tang, C. C., Luo, F., Li, X. Y., Pei, Z. Y., Gao, J. M., and Sun, S. J. (2015). Responsiveness of sweet sorghum in yield and quality related traits to environmental factors. *Acta Agron. Sinica*. 41, 1441–1447 (in Chinses with English abstract). doi: 10.3724/SP.J.1006.2015.01612

Tang, C., Yang, X., Chen, X., Ameen, A., and Xie, G. (2018). Sorghum biomass and quality and soil nitrogen balance response to nitrogen rate on semiarid marginal land. *Field Crops Res*. 215, 12–22. doi: 10.1016/j.fcr.2017.09.031

Tew, T., L., Cobill, R. M., and Richard, E. P. (2008). Evaluation of sweet sorghum and sorghum × sudangrass hybrids as feedstocks for ethanol production. *Bioenerg. Res*. 1, 147–152. doi: 10.1007/s12155-008-9013-y

Vasilakoglou, I., Dhima, K., Karagiannidis, N., and Gatsis, T. (2011). Sweet sorghum productivity for biofuels under increased soil salinity and reduced irrigation. *Field Crop Res*. 120, 38–46. doi: 10.1016/j.fcr.2010.08.011

Wei, M., Zhu, W., Xie, G., Lestander, T. R., Wang, J., Xiong, S., et al. (2014). Ash composition in cassava stems originating from different locations, varieties, and harvest times. *Energ. Fuels*. 28, 5086–5094. doi: 10.1021/ef5009693

Weng, J. K., Li, X., Bonawitz, N. D., and Chapple, C. (2008). Emerging strategies of lignin engineering and degradation for cellulosic biofuel production. *Curr. Opin. Biotechnol.* 19, 166–172. doi: 10.1016/j.copbio.2008.02.014

Wortmann, C. S., Liska, A. J., Ferguson, R. B., Lyon, D. J., Klein, R. N., and Dweikat, I. (2010). Dryland performance of sweet sorghum and grain crops for biofuel in Nebraska. *Agron. J.* 102, 319–326. doi: 10.2134/agronj2009.0271

Xie, G. H. (2012). Progress and direction of non-food biomass feedstock supply research and development in China. *J. Chin. Agr. Univ*. 17, 1–19 (in Chinses with English abstract).

Yu, J. L., Zhang, X., and Tan, T. W. (2008). Ethanol production by solid state fermentation of sweet sorghum using thermotolerant yeast strain. *Fuel Process. Technol.* 89, 1056–1059. doi: 10.1016/j.fuproc.2008.04.008

Zegada-Lizarazu, W., and Monti, A. (2012). Are we ready to cultivate sweet sorghum as a bioenergy feedstock? A review on field management practices. *Biomass Bioenerg.* 40, 1–12. doi: 10.1016/j.biombioe.2012.01.048

Zhang, C. X., Xie, G. D., Li, S. M., Ge, L. Q., and He, T. T. (2010). The productive potentials of sweet sorghum ethanol in China. *Appl. Energ.* 87, 2360–2368. doi: 10.1016/j.apenergy.2009.12.017

Zhao, Y. L., Dolat, A., Steinberger, Y., Wang, X., and Osman, A. (2009). Biomass yield and changes in chemical composition of sweet sorghum cultivars grown for biofuel. *Field Crop Res*. 111, 55–64. doi: 10.1016/j.fcr.2008.10.006

Zhao, Y. L., Steinberger, Y., Shi, M., Han, L. P., and Xie, G. H. (2012). Changes in stem composition and harvested produce of sweet sorghum during the period from maturity to a sequence of delayed harvest dates. *Biomass Bioenerg.* 39, 261–273. doi: 10.1016/j.biombioe.2012.01.020

Zhuang, D. F., Jiang, D., Liu, L., and Huang, Y. H. (2011). Assessment of bioenergy potential on marginal land in China. *Renew. Sust. Energ. Rev.* 15, 1050–1056. doi: 10.1016/j.rser.2010.11.041

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Plant Science

ORIGINAL RESEARCH
published: 19 July 2017
doi: 10.3389/fpls.2017.01289

A Diagnosis of Biophysical and Socio-Economic Factors Influencing Farmers' Choice to Adopt Organic or Conventional Farming Systems for Cotton Production

Amritbir Riar[1]*, Lokendra S. Mandloi[2], Randhir S. Poswal[3,4], Monika M. Messmer[5] and Gurbir S. Bhullar[1]

[1] Department of International Cooperation, Research Institute of Organic Agriculture, Frick, Switzerland, [2] bioRe Association India, Khargone, India, [3] Central Soil Salinity Research Institute, Karnal, India, [4] Agricultural Extension Division, Indian Council of Agricultural Research, New Delhi, India, [5] Department of Crop Science, Research Institute of Organic Agriculture, Frick, Switzerland

Organic agriculture is one of the most widely known alternative production systems advocated for its benefits to soil, environment, health and economic well-being of farming communities. Rapid increase in the market demand for organic products presents a remarkable opportunity for expansion of organic agriculture. A thorough understanding of the context specific motivations of farmers for adoption of organic farming systems is important so that appropriate policy measures are put in place. With an aim of understanding the social and biophysical motivations of organic and conventional cotton farmers for following their respective farming practices, a detailed farm survey was conducted in Nimar valley of Madhya Pradesh state in central India. The study area was chosen for being an important region for cotton production, where established organic and conventional farms operate under comparable circumstances. We found considerable variation among organic and conventional farmers for their social and biophysical motivations. Organic farmers were motivated by the sustainability of cotton production and growing safer food without pesticides, whereas conventional farmers were sensitive about their reputation in community. Organic farmers with larger holdings were more concerned about closed nutrient cycles and reducing their dependence on external inputs, whereas medium and small holding organic farmers were clearly motivated by the premium price of organic cotton. Higher productivity was the only important motivation for conventional farmers with larger land holdings. We also found considerable yield gaps among different farms, both under conventional and organic management, that need to be addressed through extension and training. Our findings suggest that research and policy measures need to be directed toward strengthening of extension services, local capacity building, enhancing availability of suitable inputs and market access for organic farmers.

Keywords: organic cotton, motivational factors, biophysical factors, socio-economic factors

Edited by:
José Manuel Mirás-Avalos,
Centro de Edafología y Biología
Aplicada del Segura (CSIC), Spain

Reviewed by:
Mongi Sghaier,
Institute of Arid land, Tunisia
Jo Smith,
Organic Research Centre,
United Kingdom

****Correspondence:***
Amritbir Riar
amritbir.riar@fibl.org

Specialty section:
This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Plant Science

Received: *24 April 2017*
Accepted: *07 July 2017*
Published: *19 July 2017*

Citation:
Riar A, Mandloi LS, Poswal RS, Messmer MM and Bhullar GS (2017) A Diagnosis of Biophysical and Socio-Economic Factors Influencing Farmers' Choice to Adopt Organic or Conventional Farming Systems for Cotton Production. Front. Plant Sci. 8:1289. doi: 10.3389/fpls.2017.01289

INTRODUCTION

Global population is projected to reach 11 billion by the end of 21st century (Alexandratos and Bruinsma, 2012) further escalating the multifaceted challenges ahead of modern agricultural systems. In a scenario where nearly 1 billion people are currently undernourished (FAO, 2011) our agricultural system needs to ensure the provision of sufficient and affordable nutrition for everyone. About a billion hectares of additional cropland are needed by 2050 to meet the projected increase of 70–110% in global food demand using contemporary farming practices (Tilman et al., 2001; Bruinsma, 2009). Most of the additional land will come from developing countries mainly in the tropics, which will inhabit more than half of the world's population by the middle of this century (Edelman et al., 2014). This makes further intensification of agro-ecosystems imminent, which needs to be brought in such a way that ecological balance of our planet is maintained (Andres and Bhullar, 2016).

In recent decades, fossil based input-intensive industrial agricultural technologies have been widely recognized as being unsustainable over the long-run (Pingali, 2012). Moreover, global food system has increasingly faced the impacts of escalating intensity of climatic extremes (Nelson et al., 2009) as well as economic uncertainties (Kastner et al., 2012). This has led to stronger calls for transformation of our agricultural system using holistic farming practices based on ecological principles. Several different alternative farming approaches have been put forward in different parts of the world from time to time with varying degree of success. Organic agriculture is one of the most widely known alternative agricultural production systems advocated for its benefits to soil, environment, health and economic condition of farming communities (Mäder et al., 2002; Badgley et al., 2007; Forster et al., 2013).

Steep growth in organic markets has resulted in global demand for organic products surpassing the total production (Sahota, 2016; Willer and Lernoud, 2016). Until recently, organic market was primarily dominated by the developed countries, where prosperous consumers can afford to pay premium prices for organic products. Organic sector in the developing countries has largely been export oriented, however, with rapid economic development domestic organic markets are currently seeing significant expansion in the emerging economies (Sirieix et al., 2011). There have been strong calls for mainstreaming of organic agriculture in some of the developing countries as well (Scialabba, 2000; UNCTAD-UNEP, 2008) and in some cases governments in different parts of the world have implemented pro-organic policies (Kolanu and Kumar, 2007; FAO, 2011; Wai, 2016). This presents a remarkable opportunity, particularly for small and medium holding farmers in developing countries (Rundgren, 2006). To fully utilize the available potential, appropriate implementation of policy measures is necessary, which demands a context specific understanding of available scenarios. For instance, the adoption rates of organic farming practices may vary among farmers depending upon various factors, including those of biophysical and socio-economic nature. Understanding the motivation of farmers for adoption of their specific set of agricultural management practices is of crucial importance to design suitable policy measures.

Since ancient times, India has been an important exporter of cotton. India regained its position as world's largest producer of cotton in 2014–2015, as Indian farmers consistently produced over 6 million tons of cotton lint in 2013–2015. A dramatic change in the age old cotton cultivation practices in India happened in the second half of 20th century as the indigenous or 'Desi' varieties (*Gossypium arboreum*) were first replaced by American cotton (*Gossypium hirsutum*) varieties and hybrids and subsequently by genetically modified Bt-cotton. Because of the resistance to cotton bollworms and hence reduced pesticide usage, Bt-cotton was adopted by farmers relatively quickly after its first release in 2002 (Finger et al., 2011; James, 2011; Krishna and Qaim, 2012; Qaim and Kouser, 2013). Today, more than 95% of cotton produced in India is Bt-cotton, yet the impact of Bt-cotton adoption on farmers' livelihood and environment is debated (Stone, 2011; Kathage and Qaim, 2012). Moreover, many reports of bollworms attaining resistance to Bt-toxin and emergence of secondary pests question the sustainability of this technology (Tabashnik, 1994; Luttrell et al., 2004; Bagla, 2010; Downes et al., 2010, 2016).

The productivity of cotton is limited by the following external factors: Scale of production, level of research support, local ginning capacity, access to quality seed, access to irrigation, access to timely inputs, production costs, price paid for seed cotton, access to credit, timely payment for the crop and availability of season-long farmer training (Page and Ritchie, 2009). The biggest sustainability challenge in conventional cotton production remains the need for high inputs of agrochemicals, many of which are known for their adverse effects on human health and potential harm to the environment (Page and Ritchie, 2009; Bachmann, 2012). Since most of the cotton produced in India is grown by smallholder, subsistence farmers usually with land holdings of less than one hectare, capital intensive high input farming is not the most suited choice for them. Organic production offers a suitable alternative to such farmers with potential advantages of lower expenses for farm inputs, healthier soils and environment as well as competitive gross margins (Rajendran et al., 2000; Lakhal et al., 2008; Forster et al., 2013). Despite the fact that only less than 5% of cotton produced in India is certified organic (Stone, 2011; Kathage and Qaim, 2012), India is still leading the global organic cotton production, as it contributed 66.9% of the worldwide production in 2014–2015 (Truscott et al., 2016). The global production of organic cotton saw a rapid growth from 2006 to 2010, which started to decline from 2011 onward (Truscott et al., 2016). With a steep increase in demand of organic fiber (Truscott et al., 2016), it is important to safeguard and increase the production of organic cotton in a sustainable manner.

Although India is a significant producer of organic crops, the bulk of organic production has been largely targeted at export markets. The share of domestic market is steadily increasing owing to the recent economic developments and consumer awareness (Chandrashekar, 2010). However, there is a strong need for development and implementation of appropriate policy measures considering the choices and motivations of farmers. This study was aimed at diagnosing the biophysical and socio-economic factors influencing the adoption of organic and conventional management practices by the cotton farmers in order to facilitate appropriate policy development. We hypothesized that the motivation of farmers for adoption of conventional or organic farming systems differs depending upon their awareness level, social perceptions, availability of resources and perceived profitability.

MATERIALS AND METHODS

Study Region

This study was conducted in the Nimar valley of Madhya Pradesh state in central India, which is an agriculturally important region. In the study area, cotton is cultivated as a major cash crop, in rotation with other crops such as cereals, vegetables, and legumes (Myers and Stolton, 1999; Eyhorn et al., 2007). Studies comparing organic and conventional farming systems in this region have showed that performance of both the systems is somewhat comparable to each other (Eyhorn et al., 2007; Forster et al., 2013; Helfenstein et al., 2016). However, cotton yields in general are low and variable in Nimar valley and often do not reach the attainable levels on several farms of the region. This unique situation where conventional and contemporary organic agricultural systems are existing in parallel in a society with wide economic disparities offers a rigorous platform to understand the biophysical and socio-economic motivational characters of farmers. The main aim of this study was to identify social and biophysical motivational characters controlling rational decision of farmers to opt for either organic or conventional agricultural system at farm level.

Farm Survey

During the cotton season of 2015 (May to December), a detailed structured survey of organic and conventional cotton farms was conducted in the cotton growing region of west Nimar. Survey questions were standardized in preliminary focussed group discussions with farmers, extension workers, research staff and other stakeholders using the joint innovation platform of the Research Institute of Organic Agriculture (FiBL) and bioRe Association (Andres et al., 2016). For structured survey, individual interviews were conducted at 60 organic and 60 conventional farms randomly selected from five different cotton growing pockets/clusters of west Nimar. Each farm was treated as a single operational unit and the farmer responsible for decision-making was interviewed. Farmers were selected solely based upon their farming practices, irrespective of farm size, soil type, education, income or any other demographic factors. In order to identify the social, biophysical and economic motivational factors behind adoption of a particular farming system (organic/conventional) by the farmers, the survey questionnaire included a section with a number of statements relating to views on farming practices and their sustainability. The farmers were asked to mark the category best describing their level of agreement with the statement (not, little, quite, very, and extremely). Additionally, survey respondents (farmers) had the possibility to add their own statements regarding major limiting factors for cotton production, in their preference to grow organic cotton and switching from conventional to organic. Upon careful consideration of each of such statements, they were grouped into thematically relevant categories.

For statistical analysis, farmers were further grouped according to size of their land holdings, in order to broadly represent different socio-economic categories. They were grouped into small (<2 ha), medium (2–4 ha), and large (>4 ha) holding farmers, with the small scale farmers recognized as being asset-poor (Singh et al., 2010; Coventry et al., 2015). Upon further subgrouping it was found that the number of respondents was too low in certain sub-categories to arrive at statistically sound conclusions per group. However, the number of respondents are sufficiently large to be able to discern issues and emerging trends. The survey targeted whole farm information on cotton crop management practices (including variety selection, fertilizer management, weed and pest management, number of picking) as well as the information on farmer demography and attitudes. Each farmer was personally visited by one of the designated staff members of bioRe extension team. The staff members were instructed in survey data compilation, to safeguard standardized survey data collection and preparation. Informed consent was obtained from all individual participants included in the study. The data were collected in an Excel document and to derive inferences Principal Component Analysis (PCA) were conducted on this data set.

Principal Component Analysis

To do the PCA, the number of farmers selecting each of the limiting factors divided by the total number of farmers within each farm size group was calculated as a percentage using JMP (© SAS Institute Inc.) (Goupy and Creighton, 2007). Farming practices and farm size were included as factors and all the surveyed social and management related limiting factors were included as variables, and covariance was selected as the matrix type.

RESULTS AND DISCUSSION

Profile of Respondents – Gender, Age, Education and Experience

Survey results show that farming is a means of livelihood in Nimar valley area and predominantly a male dominant profession as 86% of the total farm units surveyed were led by male farmers (**Table 1**). Interestingly, the proportion of farms managed by female farmers as operational head of farm was higher in organic farms group compared to conventional farms (17% v. 11%). Furthermore, the farm size showed a distinguishing feature, since the proportion of farms managed by female farmers was higher on farms with large land holdings both on conventional and organic farms (**Table 1**). This result is of particular significance since women are believed to be the quiet drivers of change toward more sustainable production systems and healthier diets (Altieri and Koohafkan, 2008). Women comprise of more than 40% of the agricultural labor

TABLE 1 | Profile of surveyed respondents for gender, age, education, farming experience and land holding.

Farming practices	Conventional				Organic			
	Small	Medium	Large	Overall	Small	Medium	Large	Overall
Male %	100.0	89.3	77.8	88.3	90.9	94.3	64.3	86.7
Female %	0.0	10.7	22.2	13.2	9.1	5.7	35.7	13.3
Average age (Years)	47.6	40.9	44.2	44.2	44.5	46.8	44.9	45.4
Education < 5 year (%)	71.4	71.4	38.9	61.7	72.7	60.0	28.6	55.0
Education > 5 year (%)	28.6	28.6	61.1	38.3	27.3	40.0	71.4	45.0
Experience in farming (Years)	17.8	23.4	28.3	23.2	23.2	21.7	23.8	22.9
Average land holding (ha)	1.25	2.91	7.60	3.9	1.30	2.77	5.46	3.2

force in developing countries and up to 50% in Asia and sub-Saharan Africa. In recent decades, development agencies and policy advocates have been emphasizing that women could increase the farm productivity by 20–30%, if they have the same access to productive resources as men (Lastarria-Cornhiel, 2006; Altieri and Koohafkan, 2008). However, in an extensive review of available literature, Doss (2015) found men and women to be equally productive, given the access to similar resources. In our study, the productivity of organic farms operated by females was statistically similar, i.e., 1410 ± 161 kg ha^{-1} and 1396 ± 121 kg ha^{-1} of organic farms operated by female and male farmers, respectively. Similar to organic farms, productivity of conventional farms led by male (1819 ± 123 kg ha^{-1}) and female (1792 ± 327 kg ha^{-1}) farmers also did not differ. Like all other faces of life, the participation of women at decision-making capacities has also increased in agriculture also in developed countries. According to a report by the US Department of Agriculture's Economic Research Service, farms operated by women increased to 14% in 2007, up from 5% in 1978 (Hoppe and Korb, 2013). Some studies in developed countries (mainly Europe) have tried to generalize the differences among organic and conventional farmers based on their age, education, farming experience and land holding. For instance, Rigby and Cáceres (2001) characterized organic farmers in United Kingdom (UK) as typically smaller in terms of land holding with better education and of younger age with urban background and little experience. In our study, the organic and conventional farmers in Nimar valley did not differ for these characteristics. Average age of the farm head came out to be 44 years for conventional farmers and 45 years for organic farmers. The oldest conventional respondent was 75 years old and youngest was 24 years old whereas among the organic farmers, oldest respondent was 70 years old and youngest respondent was 27 years old.

Survey also showed that education was low in Small and Medium land holding farmers in both conventional and organic farms. On an average, only 38.3% of conventional farmers and 45.0% of organic farmers had more than 5 years of formal education. Level of education showed positive relationship with the land holding as within large land holding farmers, 61.1% of conventional farmers and 71.4% of organic farmers had more than 5 year of formal education. All surveyed farmers showed similar level of experience in farming (average 23 years; range 18–28 years). Reported gross agricultural income ha^{-1} was comparable across farm sizes and farming systems (**Figure 1**). On conventional farms, median income per unit of land decreased as the land holding increased (**Figure 1**), whereas level of income per unit of land remained unrelated to landholding of organic farmers and did not vary much among the farm sizes.

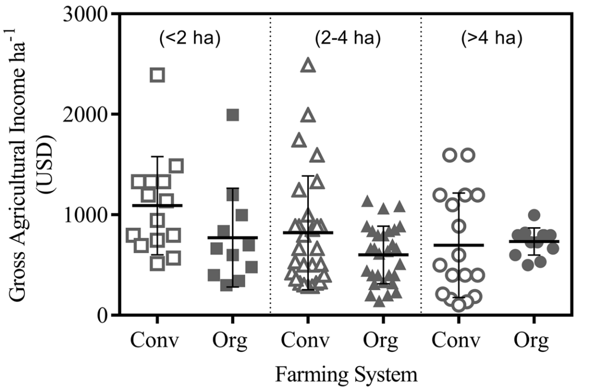

FIGURE 1 | Gross agricultural income ha^{-1} of organic (Org) and conventional (Conv) farmers (1 USD = 62 INR).

Farmers' View on Major Limiting Factors of Cotton Production in Nimar Valley

In an open ended question, conventional and organic farmers were asked about their major concerns on cotton production in Nimar valley. Climatic uncertainty, pest and disease attack were the main concerns of conventional and organic farmers (**Figure 2**). The concerns about climatic uncertainty were raised by proportionately higher number of organic farmers compared to conventional farmers. We found that organic farmers had limited options and capacities for production of botanical extracts to deal with pest and disease incidences. Since seasonal variations have a high degree of influence on frequency and magnitude of pest and disease attacks, the concerns of organic farmers regarding climatic uncertainty indirectly relate to pest and disease attack. Similar concern were also observed in United States by Organic Trade Association (Organic Trade Association, 2015). The conventional farmers interpreted climatic uncertainty in terms of rainfall pattern and distribution throughout the cotton growing season. Low production was the other main

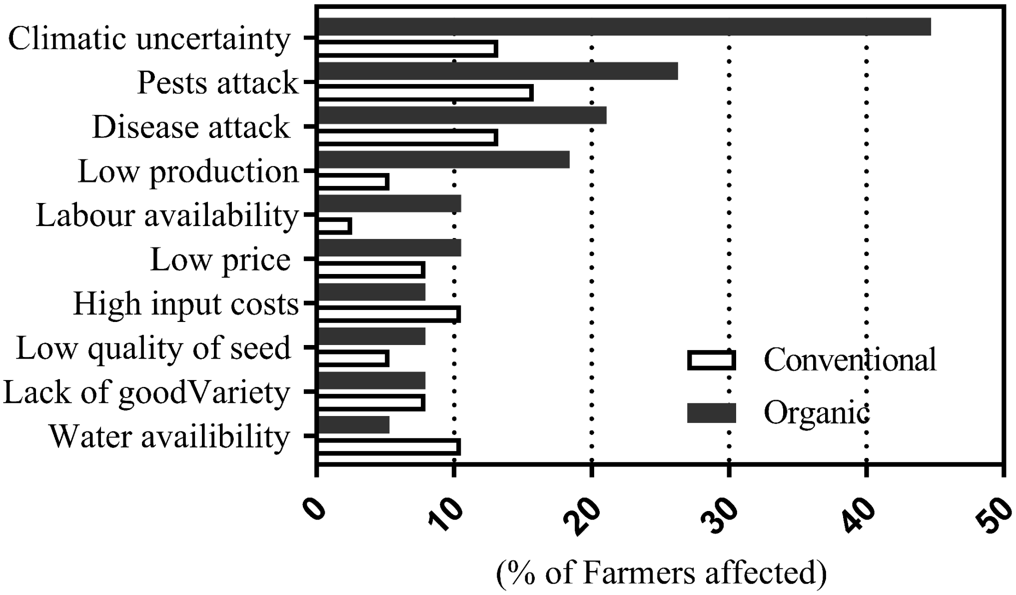

FIGURE 2 | Organic and conventional farmers' views on major limiting factors of cotton production in Nimar valley.

concern raised by organic farmers in direct open ended questions, however, elaborated data analysis showed that median yield and yield variation was similar between organic and conventional farms (**Figure 2**). This also shed light on the assumption of satisfactory yield levels, i.e., different farmers could perceive same yield levels as being 'high' or 'low' depending upon their perspective and awareness. Nevertheless, it is also evident that competitive performance of different agriculture systems vary in different environments and crops; Birkhofer et al. (2008) found that organic system yield 23% lower than conventional system whereas (Reganold et al., 2001) found that organic and conventional system perform similar in apple production.

Labor availability was also a major concern amongst organic farmers compared to conventional. The local farmers perceived that mechanical operations can only be performed on conventional farms whereas organic farming has to be done in more traditional ways. More labor requirement in organic was mainly associated to hand weeding and spraying of the botanical extracts. Lakhal et al. (2008) noted that the organic cotton farmers use 10 times more hired labor than the conventional cotton farmers. Noticeably, the concerns about low price, high input costs, poor quality seed (Hillocks and Kibani, 2002; Page and Ritchie, 2009), lack of high yielding varieties (Page and Ritchie, 2009), and non-availability of water were similar in both organic and conventional farmers.

Cotton Yield

A number of factors could influence yield of cotton, crop management practices being the prominent one. Farm size could be a major factor influencing the decision-making and effective implementation of adequate management, whereas irrigation facilities and soil type could be limiting factors for water and nutrient supply to the cotton crop. Farmers were asked to report cotton yield in last 3 years (2012, 2013, and 2014). Means of the reported yields were analyzed against the above-mentioned limiting factors to understand the cotton productivity scenario for both organic and conventional farms in Nimar valley. Analysis showed that the influence of farm size on cotton yield in general was statistically insignificant (**Figure 3A**). The average yield of cotton crop was 1270 ± 383 kg ha^{-1} and 1926 ± 515 kg ha^{-1} on small organic and conventional farms, respectively. Medium sized organic and conventional farms showed comparable cotton yields (1473 ± 253 kg ha^{-1} and 1556 ± 299 kg ha^{-1}) with very little variability among the farms. Yield on large size organic farms was 1315 ± 351 kg ha^{-1} compared to 1961 ± 476 kg ha^{-1} on conventional large size farms but both groups did not differ significantly to each other. Most of the surveyed cotton farms had irrigation facilities (**Figure 3B**). The median yield of irrigated cotton organic farms was 1430 ± 121 kg ha^{-1} compared to 1768 ± 115 kg ha^{-1} for irrigated conventional farms. Organic farms with two soil types had lower yield (1239 ± 99 kg ha^{-1}) compared to conventional farms that have fields with both soil types (2107 ± 247 kg ha^{-1}) (**Figure 3C**). All other groups based on different soil types showed similar yield levels.

Findings from the long-term farming systems comparison experiment located in the same region as this study (Nimar valley) showed that cotton yield in organic production system matched those of conventional production system as soon as the conversion period was over Forster et al. (2013). A farm survey conducted in the same region also showed comparable cotton yields of organic and conventional farms (1459 ± 83 kg ha^{-1} vs. 1400 ± 67 kg ha^{-1}) in 2003 and (1237 ± 105 kg ha^{-1} vs. 1166 ± 70 kg ha^{-1}) 2004, respectively (Eyhorn et al., 2007). Similarly, in a recent farms survey comparable yields of wheat were found on organic and

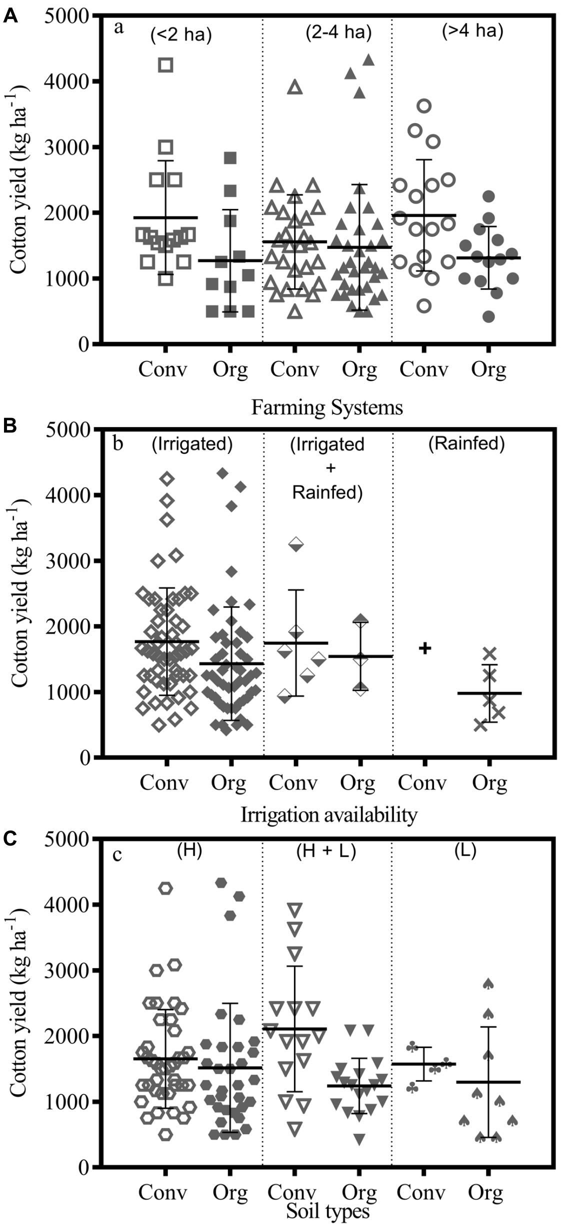

FIGURE 3 | Cotton yield of conventional (Conv) – open circles – and organic farms (Org) – closed circles – in relation to **(A)** farm size, **(B)** irrigation availability, and **(C)** soil types, where each circles represent the reported averaged cotton yield for last 3 years; H: Heavy cotton soil (Vertisol); L: Light soil (Inceptisols and entisols).

conventional farms (Helfenstein et al., 2016). In our study, the analysis of three key factors (farm size, irrigation facilities, and soil type) showed that the range of variation among the farms was far-flung, hence it could be concluded that cotton yield gets limited by other factors before it comes to the level where it can be limited primarily by water and soil nutrients. In each category, there were some farms with relatively high productivity as well as with poor productivity. Widespread variation in cotton yield among the farms also indicates that the first step to increase yield would be to improve management practices of cotton crop at individual farm. Therefore, farmers' knowledge need to be strengthened to improve their understanding and skills (Misiko et al., 2011).

Farmers' Motivational Characters behind Farming Practices

While there are no differences among organic and conventional farmers with regard to their age, education, experience and farm size, there must be some other factors influencing their decision to choose either organic or conventional way of farming. We used principle component analysis (PCA) to identify the social, economic, and biophysical motivations of different farmers for following their respective farming practices. PCA provided an overview of the relationship of organic and conventional farming practices on different sized farms to social motivational characters of the farmers as well as to the biophysical reasons perceived by them (**Figures 4**, **5**). In the biplot figures (**Figures 4**, **5**), the axis labels indicate the extent to which the mentioned factors account for the total variation in data. The proximity of a farming system group to a particular motivational character demonstrates the agreement of the farmers in that group to the influence from that character and the length of the vector shows the degree of influence compared to other characters.

Social Motivational Characters

Analysis of survey data revealed that the motivational characters vary among farmers following specific farming practice and having different farm sizes. Besides the differences among different farm sizes, the points pertaining to organic and conventional farm groups spread into different coordinate quadrants (**Figure 4**) indicate the ideological differences among the followers of these two production systems. The first component of PCA accounting for 63.1% of the total variation, and first component + second component accounting for 85.1% of the total variation showed that these are the most common listed social motivational factors that impact on adoption of a specific management system for cotton production. Some of the social motivation factors such as perception of climate change, habitual reasons, long-term sustainability, interest to grow safer food and societal influence were more important on total variation than others as indicated by the long length of vectors in **Figure 4**. Studies conducted in Canada and United States have reported similar concerns as motivation of farmers for converting to organic, e.g., concerns over environmental impact of farming (Henning, 1994) and motivation for personal, family, or consumer health and safety (Cacek and Langner, 1986; Lockeretz and Madden, 1987; Molder et al., 1991; Henning, 1994; Hall and Mogyorody, 2001; Cranfield et al., 2010).

Long-term sustainability of cotton was the major motivation for organic farmers with larger land holdings (>4 ha). Whereas, growing safer food without pesticides and a wish to handover their land to the next generation in a better condition were expressed as main motivations by the organic farmers with

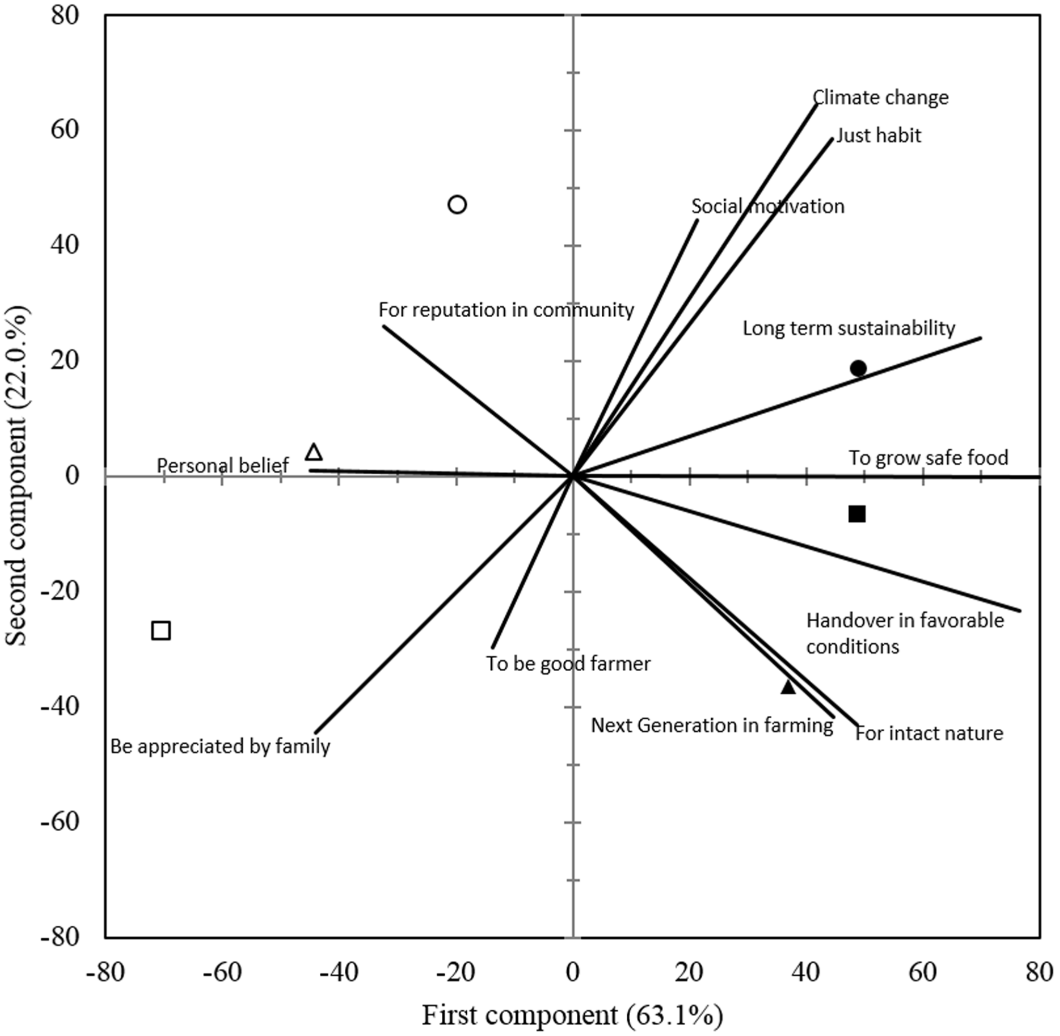

FIGURE 4 | Biplot for the principal component analysis of the respective social motivational characters of (●) large, (▲) medium, and (■) small holding organic farmers; as well as (○) large, (Δ) medium, and (□) small holding conventional farmers. Closeness of a farming system symbol to a particular motivational character confers the dominance of that motivation, whereas length of the vector line signifies the effect of that motivational character.

medium sized holdings (2–4 ha). However, it is noteworthy that only 32.3% of the surveyed organic medium holding farmers wanted their children to become farmer 1 day. Motivation of small holding (<2 ha) organic farmers was to perform agricultural practices that are favorable for an intact nature and 33.3% of them wanted their children to become farmers 1 day. In contrary to organic farmers, the motivation of conventional farmers was ambiguous. Large holding conventional farmers did not seem to derive their motivation from the mentioned social factors as indicated by the remote presence of point pertaining to this group in 2nd quadrate (**Figure 4**). The closest vector indicated that they were only concerned about their reputation in the community. Medium holding conventional farmers believed that the conventional practice was a better way of farming (personal belief). However, the small holding conventional farmers seemed to be aloof of the studied social factors and therefore, the social motivation of this farming group remains unclear. The closeness to vectors of 'personal belief' and 'appreciation from family' may suggest lack of awareness and limited risk bearing ability, preventing a shift from the existing farming practices.

Biophysical and Economic Motivational Characters

Similar to the social motivational characters, the points pertaining to organic and conventional farming groups with different farm holdings were spread into different coordinate quadrants, clearly distinguishing the biophysical motivational characters of each group. As the first and second component together account for 73.6% of the total variation, it means that the listed biophysical factors are the most common ones influencing the surveyed organic and conventional farms (**Figure 5**). Current price of cotton, avoiding the exposure to pesticides and closed

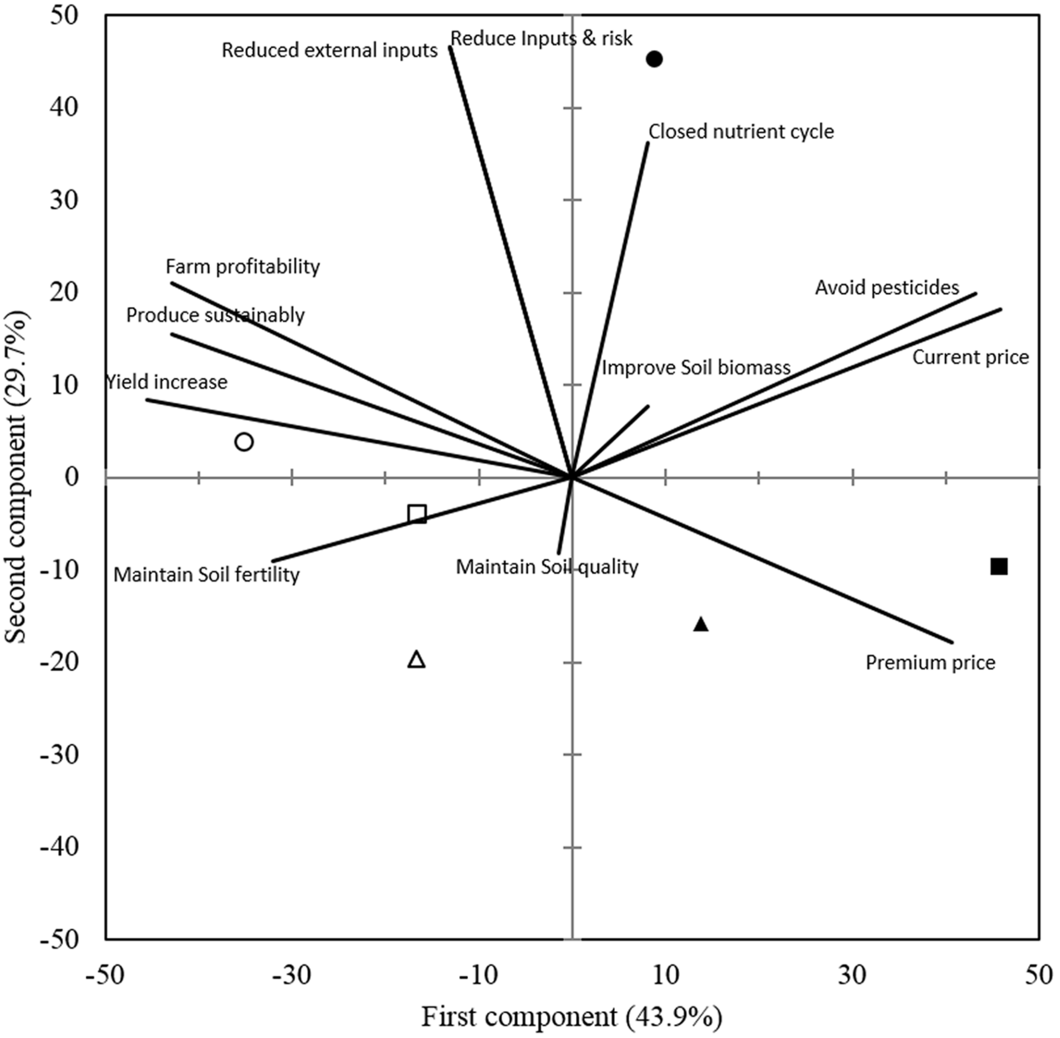

FIGURE 5 | Biplot from the principal component analysis of the biophysical factors influencing the adoption of specific practices by (●) large, (▲) medium, and (■) small holding organic farmers as well as (○) large, (Δ) medium, and (□) small holding conventional farmers.

nutrient cycles turned out to be more important factors on total variation than other ones, as indicated by their long length of vectors in the first quadrant. Reduction of the production costs and risk of ineptness by being independent of external inputs as well as the premium price were some other important factors for organic farmers. Closer review of the responses revealed that large holding organic farmers were more concerned about closed nutrient cycles to reduce their dependence on external inputs, whereas medium and small holding organic farmers were clearly motivated by the premium price of organic cotton. Results of this study as well as previously conducted studies in advanced economies reveal that profitability/financial return is gaining importance as a stronger decision-making factor in opting for organic. In a survey conducted by Henning (1994), only 9% of the study respondents indicted profitability as important factor, whereas in a survey of 2001, 56% of the respondents mentioned profitability as *very important* factor for conversion to organic agriculture (Hall and Mogyorody, 2001). On the other hand large holding conventional farmers in our study did not opt for organic agriculture as they believed that high yield was the key to success which could only be achieved by conventional practices. As in the case of social motivational factors, medium holding conventional farmers did not have any clear consideration of biophysical factors for adoption of conventional farming. Small holding conventional farmers believed that the application of fertilizers is important to improve the fertility of their soils. In addition, opportunistic decisions influenced by changed circumstances could contribute to farmers' adoption or abandoning of a specific farming system. For instance, Eyhorn et al. (2007) reported 30–40% fallback rate of organic cotton farmers to conventional practices under the influence of campaign by companies selling newly introduced Bt-cotton seed in 2003.

TABLE 2 | Main reasons of farmers to opt for organic cotton production and the proportion of farmers assigning importance to each.

Response	% farmers
Low production cost	31.6
Premium price	16.5
Cash payment	12.0
Door-step purchasing	12.0
Improves the soil health	12.0
Stable production	3.8
No wilting problem	3.0
Easy seed availability from contractor	2.3
Personal preference	2.3
Intact nature	1.5
No disease	1.5
No dependency on market	0.8
Low risk	0.8

TABLE 3 | Scenarios for shifting from conventional to organic farming practices.

Sr. No.	Response	% farmers
1	When cost benefit ratio will further decline due to high input costs related to conventional farming practices	61.9
2	When soil fertility become too low, I would opt for organic agriculture to maintain it.	23.8
3	If I get substantial support from private/govt. sector for organic	6.3
4	If there is no solution of Bt-cotton wilting	4.8
5	Higher premium price	1.6
6	When more farmer of my region will opt for organic agriculture I also will go for organic.	1.6

Preference to Grow Organic Cotton

Apart from the PCA comparing different farming groups, we also sought to find out the relative importance of different factors considered important by organic farmers for adoption of organic practices. Low production cost followed by premium price, cash payment and door-step purchasing were the main motivating factors to grow organic cotton in west Nimar valley (**Table 2**). Farmers' responses explained that financial motivation was the main driving factor for the cotton production followed by sustainability (soil health + stable production) and hassle free management of organic cotton crop. Rigby and Cáceres (2001) also identified the financial motivation and soil health as two out of four major key motivational factors for organic farming in United Kingdom.

Switching from Conventional to Organic

In contrary to organic farmers, the conventional farmers were asked about the potential circumstances under which they can switch from conventional to organic farming. Surprisingly only six key responses surfaced, which clearly showed that conventional farmers were very clear in making the comparisons about the ground situation of organic and conventional farming (**Table 3**). Cranfield et al. (2010) reviewed the literature and categorized the motivational factors for conversion into four broad themes of (a) financial issues; (b) environmental concerns; (c) philosophical motives; and (d) health and safety concerns. Out of six key potential circumstances of cotton grower for conversion four fell into first three themes [response 1, 2, 5, and 6 (**Table 3**)]. However, in-depth analysis of motivational factor revealed that even health and safety concerns are not untouched in this part of the world and remained a subconscious motivation of organic cotton growers in Nimar valley (**Figure 5**). Similar to organic farmers, main motivation of the conventional farmers for potential conversion was also to achieve economic profit either by high yield and high price, low input cost or by hassle free management (**Table 3**).

POLICY IMPLICATIONS OF INFERENCES

The findings of this study confirm our hypothesis that the motivational characteristics of farmers for adoption of conventional or organic farming systems differs depending upon their awareness level, social perceptions, availability of resources and perceived profitability. In addition, the study results provide a detailed diagnoses of the biophysical and socio-economic factors influencing the rationale behind decision of the cotton farmers to adopt organic or conventional production systems. The inferences from this study could contribute toward the development and implementation of suitable policies promoting organic/sustainable farming systems. For instance, the large variation among cotton yields achieved by both the organic and conventional farmers highlights the tremendous scope of improvement of cotton productivity. If the underperforming farms are supported to increase their production, even to the average levels, significant increase in overall production could be achieved. In some cases, the farmers (particularly the small holders) are not even aware of the potential of increasing yields by available technologies. This is an important open area to be addressed by extension and policy institutions in collaboration with research. Innovation platforms aimed at local capacity building and development of locally adapted technologies could serve as an important tool in this direction (Andres et al., 2016).

Social motivational factors vary among organic and conventional farmers, as organic farmers are motivated by the sustainability of cotton production, growing safer food without pesticides and a wish to hand over their land to their successors in favorable condition, while the major motivation of conventional farmers is their reputation in community. Considering this, incentivising the sustainable farms for ecosystem services they provide would be an important policy measure toward achieving sustainability in agricultural systems. In case of the biophysical factors, organic farmers with larger holdings are more concerned about closed nutrient cycles and reducing their dependence on external inputs, whereas medium and small holding organic farmers are clearly motivated by the premium price of organic cotton. Since 80% farmers in India are small and medium holder, financial support during the conversion period from conventional to organic production system could serve as important driver of change to bring them on board. Higher productivity is the only important motivation for conventional farmers with larger land holdings. These results suggest that it is important to close the knowledge gap by strengthening extension services. Simultaneous and continuous training of extension workers and farmers in sustainable farming practices is of high value and thus deserves due diligence. It is also important that the farmers are made aware of the scope of increasing yields and the potential of existing technologies. Creating the awareness about yield gap and yield variation among the farmers and encouraging them to achieve maximum attainable yield by using the examples of high yielding farms could be a useful approach. Efforts need to be directed at improving the timely availability of quality on-farm inputs for organic production such as seeds and pest control measures. Moreover, research efforts need to be intensified to make available locally developed technologies and improved organic practices for nutrition, plant protection as well as agronomic management. Providing suitable marketing opportunities by developing value chains for organic produce other than cash crops (organic cotton in this case) will also be important to maintain the motivation and commitment of organic farmers as well as will provide level economic ground.

ETHICS STATEMENT

This study was carried out in accordance with the internationally accepted ethical standards for social studies and was approved by the 'Farmer Advisory committee' of bioRe Association, India. All subjects (interviewed farmers) gave written and informed consent. A formal ethics approval for this study was not needed as per our Institutional guidelines and per the relevant Indian regulations and laws.

AUTHOR CONTRIBUTIONS

AR and GB designed the study; LM and AR coordinated the data collection; AR analyzed the data; AR and GB prepared the first draft of manuscript; MM and RP supported the design and analysis; all authors revised the manuscript.

FUNDING

This work was financially supported by the Coop Sustainability Fund and was carried out using the platform of SysCom program that is jointly funded by Biovision Foundation for Ecological Development, Coop Sustainability Fund, Liechtenstein Development Service (LED), and the Swiss Agency for Development and Cooperation (SDC).

ACKNOWLEDGMENTS

We thank the extension team of bioRe India Ltd. for their support in reaching the farmers for interviews. We also acknowledge and appreciate the cooperation by the cotton farmers of Nimar valley.

REFERENCES

Alexandratos, N., and Bruinsma, J. (2012). *World Agriculture Towards 2030/2050: The 2012 Revision. No. 12-03.* Rome: Food Agriculture Organization of the United Nations (FAO).

Altieri, M. A., and Koohafkan, P. (2008). *Enduring Farms: Climate Change, Smallholders and Traditional Farming Communities*. Penang: Third World Network (TWN).

Andres, C., and Bhullar, G. S. (2016). Sustainable intensification of tropical agro-ecosystems: need and potentials. *Front. Environ. Sci.* 4:5. doi: 10.3389/fenvs.2016.00005

Andres, C., Mandloi, L. S., and Bhullar, G. S. (2016). "Sustaining the supply of White Gold: the case of SysCom innovation platforms in India," in *Innovation Platforms for Agricultural Development: Evaluating the Mature Innovation Platforms Landscape*, eds I. Dror, J. Cadilhon, M. Schut, M. Misiko, and S. Maheshwari (London: Routledge), 133–150.

Bachmann, F. (2012). Potential and limitations of organic and fair trade cotton for improving livelihoods of smallholders: evidence from Central Asia. *Renew. Agric. Food Syst.* 27, 138–147. doi: 10.1017/S1742170511000202

Badgley, C., Moghtader, J., Quintero, E., Zakem, E., Chappell, M. J., Aviles-Vazquez, K., et al. (2007). Organic agriculture and the global food supply. *Renew. Agric. food Syst.* 22, 86–108. doi: 10.1017/S1742170507001640

Bagla, P. (2010). Hardy cotton-munching pests are latest blow to GM crops. *Science* 327:1439. doi: 10.1126/science.327.5972.1439

Birkhofer, K., Bezemer, T. M., Bloem, J., Bonkowski, M., Christensen, S., Dubois, D., et al. (2008). Long-term organic farming fosters below and aboveground biota: implications for soil quality, biological control and productivity. *Soil Biol. Biochem.* 40, 2297–2308. doi: 10.1016/j.soilbio.2008.05.007

Bruinsma, J. (2009). "The Resource Outlook to 2050: By how much do land, water and crop yields need to increase by 2050?," in *Proceedings of the Expert Meeting on How to Feed the World in 2050, June 24–26,* Rome.

Cacek, T., and Langner, L. L. (1986). The economic implications of organic farming. *Am. J. Altern. Agric.* 1, 25–29. doi: 10.1017/S0889189300000758

Chandrashekar, H. M. (2010). Changing scenario of organic farming in India: an overview. *Int. NGO J.* 5, 34–39.

Coventry, D. R., Poswal, R. S., Yadav, A., Riar, A. S., Zhou, Y., Kumar, A., et al. (2015). A comparison of farming practices and performance for wheat production in Haryana, India. *Agric. Syst.* 137, 139–153. doi: 10.1016/j.agsy.2015.04.003

Cranfield, J., Henson, S., and Holliday, J. (2010). The motives, benefits, and problems of conversion to organic production. *Agric. Hum. Values* 27, 291–306. doi: 10.1007/s10460-009-9222-9

Doss, C. R. (2015). *Women and Agricultural Productivity: What Does the Evidence Tell Us?* Haven, CT: Economic Growth Center.

Downes, S., Mahon, R. J., Rossiter, L., Kauter, G., Leven, T., Fitt, G., et al. (2010). Adaptive management of pest resistance by *Helicoverpa* species (Noctuidae) in Australia to the Cry2Ab Bt toxin in Bollgard II® cotton. *Evol. Appl.* 3, 574–584. doi: 10.1111/j.1752-4571.2010.00146.x

Downes, S., Walsh, T., and Tay, W. T. (2016). Bt resistance in Australian insect pest species. *Curr. Opin. Insect Sci.* 15, 78–83. doi: 10.1016/j.cois.2016.04.002

Edelman, A., Gelding, A., Konovalov, E., McComiskie, R., Penny, A., Roberts, N., et al. (2014). *State of the Tropics 2014 Report*. Townsville City, QLD: James Cook University.

Eyhorn, F., Ramakrishnan, M., and Maeder, P. (2007). The viability of cotton-based organic farming systems in India. *Int. J. Agric. Sustain.* 5, 25–38. doi: 10.1080/14735903.2007.9684811

FAO (2011). *The State of Food Insecurity in the World. How Does International Price Volatility Affect Domestic Economies and Food Security?* Rome: Food Agriculture Organization of the United Nations (FAO).

Finger, R., El Benni, N., Kaphengst, T., Evans, C., Herbert, S., Lehmann, B., et al. (2011). A meta analysis on farm-level costs and benefits of GM crops. *Sustainability* 3, 743–762. doi: 10.3390/su3050743

Forster, D., Andres, C., Verma, R., Zundel, C., Messmer, M. M., and Mäder, P. (2013). Yield and economic performance of organic and conventional cotton-based farming systems - results from a field trial in India. *PLoS ONE* 8:e81039. doi: 10.1371/journal.pone.0081039

Goupy, J., and Creighton, L. (2007). *Introduction to Design of Experiments with JMP Examples*, 3rd Edn. Cary, NC: SAS Institute Inc.

Hall, A., and Mogyorody, V. (2001). Organic farmers in Ontario: an examination of the conventionalization argument. *Sociol. Ruralis* 41, 399–422. doi: 10.1111/1467-9523.00191

Helfenstein, J., Müller, I., Grüter, R., Bhullar, G., Mandloi, L., Papritz, A., et al. (2016). Organic wheat farming improves grain Zinc concentration. *PLoS ONE* 11:e0160729. doi: 10.1371/journal.pone.0160729

Henning, J. (1994). "Economics of organic farming in Canada," in *The Economics of Organic Farming. An International Perspective*, eds N. Lampkin and S. Padel (Oxfordshire: CABI), 143–160.

Hillocks, R., and Kibani, T. (2002). Factors affecting the distribution, incidence and spread of Fusarium wilt of cotton in Tanzania. *Exp. Agric.* 38, 13–27. doi: 10.1017/S0014479702000121

Hoppe, R. A., and Korb, P. (2013). *Characteristics of Women Farm Operators and Their Farms*. Washington, DC: U.S. Department of Agriculture, Economic Research Service.

James, C. (2011). *Global Status of Commercialized Biotech/GM Crops: 2011*. Ithaca, NY: ISAAA.

Kastner, T., Rivas, M. J. I., Koch, W., and Nonhebel, S. (2012). Global changes in diets and the consequences for land requirements for food. *Proc. Natl. Acad. Sci. U.S.A.* 109, 6868–6872. doi: 10.1073/pnas.1117054109

Kathage, J., and Qaim, M. (2012). Economic impacts and impact dynamics of Bt (*Bacillus thuringiensis*) cotton in India. *Proc. Natl. Acad. Sci. U.S.A.* 109, 11652–11656. doi: 10.1073/pnas.1203647109

Kolanu, T. R., and Kumar, S. (2007). *Greening Agriculture in India: An Overview of Opportunities & Constraints*. Hyderabad: Food Agriculture Organization of the United Nations (FAO).

Krishna, V. V., and Qaim, M. (2012). Bt cotton and sustainability of pesticide reductions in India. *Agric. Syst.* 107, 47–55. doi: 10.1016/j.agsy.2011.11.005

Lakhal, S. Y., Sidibé, H., and H'Mida, S. (2008). Comparing conventional and certified organic cotton supply chains: the case of Mali. *Int. J. Agric. Resour. Gov. Ecol.* 7, 243–255. doi: 10.1504/ijarge.2008.018328

Lastarria-Cornhiel, S. (2006). *Women's Access and Rights to Land: Gender Relations in Tenure Issues*. Rome: International Land Coalition and International Development Research Council.

Lockeretz, W., and Madden, P. (1987). Midwestern organic farming: a ten-year follow-up. *Am. J. Altern. Agric.* 2, 57–63. doi: 10.1017/S0889189300001582

Luttrell, R. G., Ali, I., Allen, K. C., Young Iii, S. Y., Szalanski, A., Williams, K., et al. (2004). "Resistance to Bt in Arkansas populations of cotton bollworm," in *Proceedings of the 2004 Beltwide Cotton Conferences, January 6–13*, San Antonio.

Mäder, P., Fliessbach, A., Dubois, D., Gunst, L., Fried, P., and Niggli, U. (2002). Soil fertility and biodiversity in organic farming. *Science* 296, 1694–1697. doi: 10.1126/science.1071148

Misiko, M., Tittonell, P., Giller, K. E., and Richards, P. (2011). Strengthening understanding and perceptions of mineral fertilizer use among smallholder farmers: evidence from collective trials in western Kenya. *Agric. Hum. Values* 28, 27–38. doi: 10.1007/s10460-010-9264-z

Molder, P. J., Negrave, P. D., and Schoney, R. A. (1991). Descriptive analysis of Saskatchewan organic producers. *Can. J. Agric. Econ. Can. Agroecon.* 39, 891–899. doi: 10.1111/j.1744-7976.1991.tb03650.x

Myers, D., and Stolton, S. (1999). *Organic Cotton: From Field to Final Product*. London: Intermediate Technology Publications Ltd (ITP).

Nelson, G., Rosegrant, M., Koo, J., Robertson, R., Sulser, T., Zhu, T., et al. (2009). *Climate Change: Impact on Agriculture and Costs of Adaptation*. Washington, DC: International Food Policy Research Institute. doi: 10.2499/0896295354

Organic Trade Association (2015). *2013 and Preliminary 2014 U.S. Organic Cotton Production & Marketing Trends*. Available at: https://ota.com/sites/default/files/indexed_files/OTA_FiberAdvocacy_140915.pdf [accessed March 21, 2017].

Page, S., and Ritchie, B. (2009). *A Report on Better Management Practices in Cotton Production in Brazil, India, Pakistan, Benin, Burkina Faso, Cameroon, Mali, Senegal & Togo*. Geneva: Better Cotton Initiative (BCI).

Pingali, P. L. (2012). Green revolution: impacts, limits, and the path ahead. *Proc. Natl. Acad. Sci. U.S.A.* 109, 12302–12308. doi: 10.1073/pnas.0912953109

Qaim, M., and Kouser, S. (2013). Genetically modified crops and food security. *PLoS ONE* 8:e64879. doi: 10.1371/journal.pone.0064879

Rajendran, A. T. P., Venugopalan, B. M. V., and Tarhalkar, P. P. (2000). *Organic Cotton Farming in India, Technical Bulletin*. Nagpur: Central Institute for Cotton Research (CICR).

Reganold, J. P., Glover, J. D., Andrews, P. K., and Hinman, H. R. (2001). Sustainability of three apple production systems. *Nature* 410, 926–930. doi: 10.1038/35073574

Rigby, D., and Cáceres, D. (2001). Organic farming and the sustainability of agricultural systems. *Agric. Syst.* 68, 21–40. doi: 10.1016/S0308-521X(00)00060-3

Rundgren, G. (2006). *Organic Agriculture and Food Security*. Bonn: International Federation of Organic Agriculture Movements.

Sahota, A. (2016). "The Global Market for Organic Food & Drink," in *The World of Organic Agriculture 2016: Statistics and Emerging Trends*, eds H. Willer and J. Lernoud (Bonn: Research Institute of Organic Agriculture (FiBL), and International Federation of Organic Agriculture Movements (IFOAM)), 133–138.

Scialabba, N. (2000). "Factors influencing organic agriculture policies with a focus on developing countries," in *Proceedings of the 13th IFOAM Scientific Conference, Basel, August 25 - September 2, 28–31*, Basel.

Singh, S., Malik, R. K., Dhankar, J. S., Yadav, A., Garg, R., Kamboj, B. R., et al. (2010). Nutrient use pattern in the irrigated rice-wheat cropping system in the Indo-Gangetic plains of Haryana, India. *Exp. Agric.* 46, 191–209. doi: 10.1017/S0014479709991086

Sirieix, L., Kledal, P. R., and Sulitang, T. (2011). Organic food consumers' trade-offs between local or imported, conventional or organic products: a qualitative study in Shanghai. *Int. J. Consum. Stud.* 35, 670–678. doi: 10.1111/j.1470-6431.2010.00960.x

Stone, G. D. (2011). Field versus farm in Warangal: Bt cotton, higher yields, and larger questions. *World Dev.* 39, 387–398. doi: 10.1016/j.worlddev.2010.09.008

Tabashnik, B. E. (1994). Evolution of resistance to *Bacillus thuringiensis*. *Annu. Rev. Entomol.* 39, 47–79. doi: 10.1146/annurev.en.39.010194.000403

Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., et al. (2001). Forecasting agriculturally driven global environmental change. *Science* 292, 281–284. doi: 10.1126/science.1057544

Truscott, L., Tan, E., Gosai, A., Emberson, L., Lambert, N., Worley, D., et al. (2016). *Organic Cotton Market Report 2016*. O'Donnell, TX: Textile Exchange, 1–78.

UNCTAD-UNEP (2008). *Best Practices for Organic Policy. What Developing Country Governments Can Do to Promote the Organic Agriculture Sector*. Geneva: United Nations Publications.

Wai, O. K. (2016). "The world of organic agriculture: statistics and emerging trends 2016," in *Proceedings of the Organi Asia 2015*, (Bonn: Research Institute of Organic Agriculture (FiBL), and International Federation of Organic Agriculture Movements (IFOAM)), 172–181.

Willer, H., and Lernoud, J. (2016). "The world of organic agriculture 2016: current statistics," in *The World of Organic Agriculture 2016: Statistics and Emerging Trends*, eds H. Willer and J. Lernoud (Bonn: Research Institute of Organic Agriculture (FiBL), and International Federation of Organic Agriculture Movements (IFOAM)), 33–116. doi: 10.4324/9781849775991

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Environmental Science

METHODS
published: 22 March 2018
doi: 10.3389/fenvs.2018.00014

Design and Manual to Construct Rainout-Shelters for Climate Change Experiments in Agroecosystems

Dominika Kundel [1,2*†], *Svenja Meyer* [3†], *Herbert Birkhofer* [4], *Andreas Fliessbach* [1], *Paul Mäder* [1], *Stefan Scheu* [3], *Mark van Kleunen* [2,5] *and Klaus Birkhofer* [6]

[1] *Soil Sciences Department, Research Institute of Organic Agriculture (FiBL), Frick, Switzerland,* [2] *Department of Biology, University of Konstanz, Konstanz, Germany,* [3] *Animal Ecology, J.F. Blumenbach Institute for Zoology and Anthropology, University of Göttingen, Göttingen, Germany,* [4] *Product Development and Machine Elements, Faculty of Mechanical and Process Engineering, Darmstadt University of Technology, Darmstadt, Germany,* [5] *Zhejiang Provincial Key Laboratory of Plant Evolutionary Ecology and Conservation, Taizhou University, Taizhou, China,* [6] *Department of Ecology, Brandenburg University of Technology, Cottbus, Germany*

Edited by:
José Manuel Mirás-Avalos,
Universidade de Santiago de Compostela, Spain

Reviewed by:
Sven Marhan,
University of Hohenheim, Germany
Claudio Lovisolo,
Università degli Studi di Torino, Italy

***Correspondence:**
Dominika Kundel
dominika.kundel@fibl.org

[†] *These two authors share first authorship.*

Specialty section:
This article was submitted to Agroecology and Land Use Systems, a section of the journal Frontiers in Environmental Science

Received: *14 December 2017*
Accepted: *08 March 2018*
Published: *22 March 2018*

Citation:
Kundel D, Meyer S, Birkhofer H, Fliessbach A, Mäder P, Scheu S, van Kleunen M and Birkhofer K (2018) Design and Manual to Construct Rainout-Shelters for Climate Change Experiments in Agroecosystems. Front. Environ. Sci. 6:14. doi: 10.3389/fenvs.2018.00014

Climate change models predict reduced summer precipitations for most European countries, including more frequent and extreme summer droughts. Rainout-shelters which intercept part of the natural precipitation provide an effective tool to investigate effects of different precipitation levels on biodiversity and ecosystem functioning. In this study, we evaluate and describe in detail a fixed-location rainout-shelter (2.5 × 2.5 m) with partial interception of natural rainfall. We provide a complete parts list, a construction manual and detailed CAD drawings allowing to rebuild and use these shelters for rainfall manipulation studies. In addition, we describe a rainout-shelter control treatment giving the possibility to quantify and account for potential shelter artifacts. To test the rainout-shelters, we established the following three treatments each in eight winter wheat plots of the agricultural long-term farming system comparison trial DOK in Therwil (Switzerland): (1) A rainout-shelter with 65% interception of rainfall, (2) a rainout-shelter control without interception of rainfall, and (3) an ambient control. The rainout-shelter effectively excluded 64.9% of the ambient rainfall, which is very close to the a priori calculated exclusion of 65.1%. In comparison to the ambient control plots, gravimetric soil moisture decreased under the rainout-shelter by a maximum of 11.1 percentage points. Air temperature under the rainout-shelter differed little from the ambient control (−0.55°C in 1.2 m height and +0.19°C in 0.1 m height), whereas soil temperatures were slightly higher in periods of high ambient temperature (+1.02°C), but remained basically unaffected in periods of low ambient temperature (+0.14°C). A maximum edge effect of 0.75 m defined a sampling area of 1 × 1 m under the rainout-shelter. The rainout-shelters presented here, proved to sustain under heavy weather and they were well-suited to be used in agricultural fields where management operations require the removal of the rainout-shelters for management operations. Overall, the results confirmed the good performance of the presented rainout-shelters regarding rainout-shelter artifacts, predictable rain exclusion, and feasibility for experimental studies in agricultural fields.

Keywords: rainout-shelter design, summer drought, climate change, precipitation, wheat, CAD drawings

INTRODUCTION

Climate change models predict a future increase in temperature and altered precipitation regimes for Central Europe (Russo et al., 2013; Spinoni et al., 2015; EEA, 2017) as well as on a global scale (IPCC, 2014). For Switzerland, average annual precipitation is predicted to decrease by 21–28% by the end of the century, accompanied by more frequent drought events in summer (CH2011, 2011). Temperature and water availability are key drivers of ecosystem functioning and effects of these changing conditions are expected on biotic and abiotic system components (Porporato et al., 2004). Effects of altered precipitation are primarily documented from forest and grassland ecosystems (Blankinship et al., 2011), with far fewer studies from agroecosystems (Wu et al., 2011; Beier et al., 2012). Models for agricultural systems predict an increased risk of crop yield loss due to higher seasonal variation in precipitation and more frequent water shortages during the growing season (Olesen and Bindi, 2002; Falloon and Betts, 2010; Trnka et al., 2011; EEA, 2017). In order to understand how climate change affects biotic and abiotic components in agroecosystems, it is crucial to simulate such precipitation regimes under field conditions.

Field studies that experimentally alter rainfall primarily use rainout-shelters to exclude ambient precipitation from a pre-defined experimental area. One group of shelter types provides a complete or almost complete exclusion of precipitation by permanently closed roofs (Svejcar et al., 1999; Fay et al., 2000; Poll et al., 2013; Prechsl et al., 2015) or by roofs that are closing automatically during rain events (Mikkelsen et al., 2008; Parra et al., 2012). Roofs that only close during rain events minimize unintended shelter effects on the microclimate, as they are only closed for short periods of time (closed for <5% of daytime, Mikkelsen et al., 2008). Yet, these roofs do not operate during strong wind, which often coincides with rainfall events and therefore do not exclude 100% of precipitation. The need for a motor and an electricity source for each roof makes this rainout-shelter type very costly for experimental designs with replicated sites and time consuming in terms of maintenance. Fixed rainout-shelters with permanently closed roofs, on the other hand, are often suitable for long-term studies. However, a complete exclusion of precipitation by a permanent roof inevitably has effects on the microclimate, such as alterations of air temperature and photosynthetic active radiation (PAR) (Beier et al., 2012). Further, in long-term studies, complete roofs necessarily need extra irrigation systems, otherwise they do not reflect realistic conditions under climate change as predicted for the next 50–100 years in most regions of Europe.

Major problems of permanent roofs relevant for biota and ecosystem processes include in particular passive warming (Svejcar et al., 1999; Fay et al., 2000; Vogel et al., 2013) and reduced PAR (Svejcar et al., 1999; Vogel et al., 2013). Reduced air circulation under complete exclusion roofs may lower the vapor-pressure deficit (VPD) and thereby reduce evapotranspiration, which in turn lowers the water demand of plants. The combination of complete exclusion roofs with irrigation systems that recirculate the intercepted rain water back onto the plots allows for flexible control of the amount of excluded precipitation (Svejcar et al., 1999; Fay et al., 2003; Castro et al., 2010), but holds the risk of changes in water chemistry (Beier et al., 2012). Again, such systems cannot be installed without access to electricity. Side-effects due to reduced air circulation and changes in water chemistry are limited by using roofs that only partially exclude rain (Yahdjian and Sala, 2002; Gimbel et al., 2015; Canarini et al., 2016). These roofs can further be designed to exclude pre-defined amounts of precipitation (e.g., according to predicted climate scenarios) during long-term experiments (Yahdjian and Sala, 2002). These authors used V-shaped acrylic bands (**Figure 1C**), which function as gullies to lead the water away and can have varying spacing in between to exclude pre-defined amounts of rain while minimizing effects on other environmental variables.

Here, we propose a revised design of the rainout-shelters by Yahdjian and Sala (2002) for the use in arable crop fields. We inspected potential side-effects of our design and provide a parts list, a construction manual and detailed CAD drawings (computer aided design) to allow construction of such rainout-shelters. The type of acrylic glass used for our rainout-shelters is highly UV-transparent, which is a major improvement over previously used shelter designs. We tested the effect of these rainout-shelters on basic abiotic conditions in cereal fields in an agricultural long-term experiment in Switzerland (DOK Trial, Mäder et al., 2002). To disentangle intended effects of the manipulated precipitation regime from unintended artifacts of the rainout-shelters, we further established two sets of control plots. Besides undisturbed plots that received ambient precipitation, we installed a replicated set of rainout-shelters that were identical to our original rainout-shelters, but allowed all natural precipitation to reach the area under the rainout-shelter (V-bands were turned over to become Λ-bands). The partial reduction of rainfall simulated by our rainout-shelters reflects predictions of future precipitation changes during the crop growing season in Central Europe (Russo et al., 2013; Spinoni et al., 2015; EEA, 2017). Our rainout-shelters are suitable for studies in a wide range of ecosystems, including agricultural systems, as they are both stable enough to endure extreme weather events in open land and are removable to allow for management activities. It is further possible to adapt the amount of excluded rainfall according to the needs of a study by adjusting the distance between the V-bands. In this manuscript, we provide a detailed description and evaluation of the proposed rainout-shelter design and discuss the performance of rainout-shelters considering intended and unintended effects on microclimate, soil moisture and edge effects.

MATERIALS AND METHODS

Site Description and Design of Drought Manipulation Experiment

We established rainout-shelters in the "DOK" farming system trial (bioDynamic, bioOrganic, Konventionell, Mäder et al., 2002). The DOK trial has been established in 1978 by the Swiss Federal Research Station for Agroecology and Agriculture

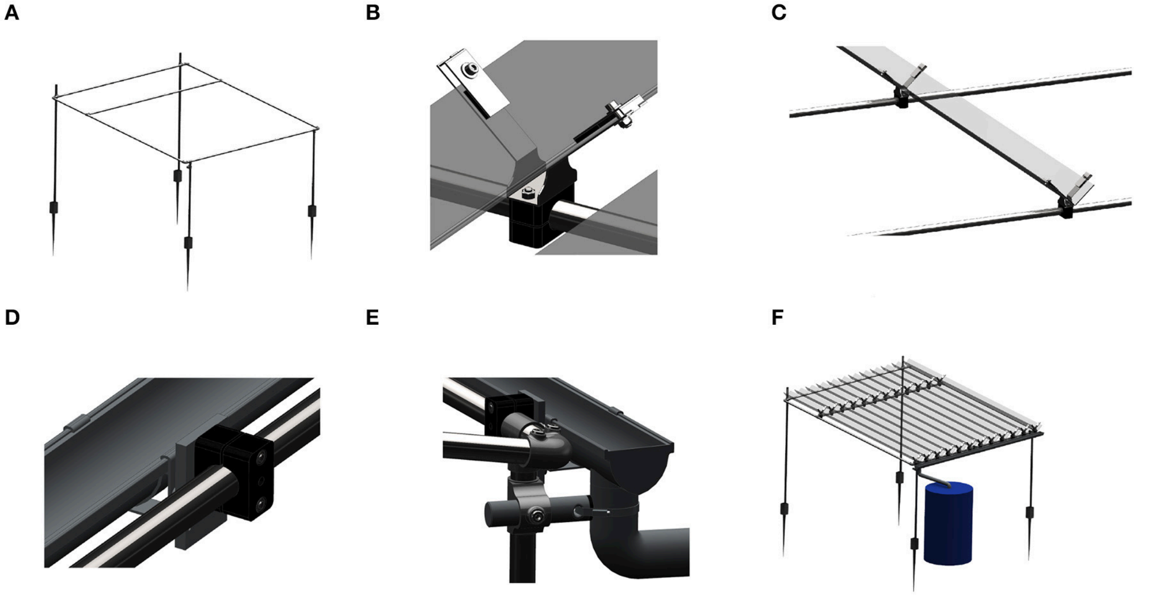

FIGURE 1 | (A) A metal frame (2.5 × 2.5 m) serves as the basic rainout-shelter construction, the shelter is anchored in the soil using drive-in sleeves; **(B)** acrylic glass bands are fixed to the steel frame by specially designed holders; **(C)** the metal frame supports V-shaped clear acrylic glass bands in which rainfall is collected; **(D)** rain gutter holder consist of pipe clamp, adapter plate, and gutter bracket (view from the rear); **(E)** a T-pipe connector with adaptor piece holds the drain pipe in place; **(F)** the final rainout-shelter with partial rainfall interception and precipitation collection.

(Zürich-Reckenholz, Switzerland) and the Research Institute of Organic Agriculture (Frick, Switzerland) to compare the production levels of arable crops under different organic and conventional farming systems (Fliessbach et al., 2007). The trial site is located in the Leimen valley near Basel, Switzerland (47°30′09.3″N 7°32′21.5″E, 300 a.s.l.) and has a slope of 3–5% in S-N-direction. Mean annual temperature at the site is 9.5°C and mean annual precipitation is 785 mm. The soil (15% clay, 70% silt, 15% sand, Fliessbach et al., 2007) at the site is a haplic luvisol on deposits of alluvial loess (Mäder et al., 2002). Soils in plots where the roofs were installed contained on average 11.9 mg organic carbon per gram of soil.

The rainout-shelter design we present here was developed in the ERA-Net Biodiversa project "SOILCLIM" (http://www.biodiversa.org/976). The main aim of SOILCLIM is to investigate links between soil biodiversity and ecosystem functioning along natural and simulated precipitation gradients and different soil organic matter (SOM) levels.

We established three treatments in four replicated winter wheat (*Triticum aestivum* L. cv. "Wiwa") plots (5 × 20 m) of two farming systems, resulting in 24 subplots. As the aim of the current study was to evaluate the general performance of the rainout-shelter, we did not differentiate between the two farming systems but treated the plots of the two systems as independent replicates ($n = 8$ plots).

The three treatments were (i) a precipitation reduction treatment with rainout-shelters (R) (ii) a rainout-shelter control treatment with a modified rainout-shelter that allowed for ambient precipitation levels to assess rainout-shelter artifacts (RC) and (iii) an untreated ambient control without any rainout-shelter (C). Treatments were established in a row, both at the near and the far end of each plot. In order to prevent mutual interference of rainout-shelter and rainout-shelter control treatments, these were never located side by side (Supplementary Figure 1). Instead, rainout-shelter and rainout-shelter control treatments were always located next to the ambient control treatment or had no adjacent treatment. Positions of treatments were randomized across the eight plots within these limitations, whereas every treatment combination occurred twice across the DOK trial. We maintained a distance between treatments as well as between treatments and field edges of at least 0.5 m. To avoid potential confounding edge effects such as lateral inflow of precipitation on our measurements, we determined all abiotic conditions only in the center of each plot (1.5 × 1 m). Approximately 2 month after rainout-shelter establishment, we quantified this edge effect by measuring gradients in soil humidity (see section Data Collection for details).

Rainout-Shelter Design

The rainout-shelters consist of a tubular steel frame (2.5 × 2.5 × 1.2–1.7 m, 6.25 m^2; **Figure 1A**) supporting 12 V-shaped clear and UV transparent acrylic glass bands (PLEXIGLAS SUNACTIVE® GS 2458, Evonik Perfomances Materials GmbH, Darmstadt, Germany). Each band had a length of 2,500 mm, an inner flange leg length of 96 mm, an angle of 90° and a thickness of 3 mm. According to Equation 1, 12 acrylic bands should exclude 65% of the ambient precipitation. The amount of intercepted precipitation can easily be adjusted by changing the number of bands (see also Yahdjian and Sala, 2002).

$$Intercepted\ precipitation\ [\%] = \frac{N * Width\ of\ band}{Shelter\ width} * 100\,\% \quad (1)$$

Equation (1): Amount of precipitation intercepted (%) by number of bands (N). For the current design: N = number of bands (here 12), width of the bands: 135.8 mm, shelter width: 2,500 mm.

In order to alter natural light conditions as little as possible, we chose a roof band material that is as permeable for the full range of PAR and transparent for most wavelengths of UV-a and -b radiation (Transmission: 380–780 nm $\geq$90%, 315 nm $\geq$80%), but is still resistant against weathering and possible damage under field conditions [for details see http://www.plexiglas.de/sites/lists/PM/DocumentsAP/222-6-PLEXIGLAS-GS-UV-durchlaessig-de.pdf (in German)]. The acrylic bands were fixed to the steel frame by custom-made holders (**Figure 1B**) on the front steel pipe and an additional central parallel steel pipe (**Figure 1C**). The rainout-shelters have a maximum height of 1.7 m and a minimum height of 1.2 m, resulting in an incline of 13°, which guarantees water run-off, but the incline can be adjusted if required. The horizontal roof parts rest on four supporting steel pipes anchored in the soil using commercially available metal drive-in sleeves (**Figure 1A**). This construction allows to temporarily remove the rainout-shelter during management actions without much effort. Shelters were located with the lower side facing west, as this is the prevailing wind direction at the study site. Water that was collected by the acrylic bands was channeled via rain gutters (**Figures 1D,E**) at the lower side of the steel frame into 310 L rain barrels (**Figure 1F**). This prevented a reflux of water onto the experimental plot under the roof and allowed to measure the amount of intercepted precipitation.

As mentioned above, we established a rainout-shelter control treatment that was identical to the rainout-shelter except that the 12 V-shaped acrylic glass bands were turned over allowing the precipitation to fall onto the plot under the rainout-shelter control. This treatment made it possible to quantify potential artifacts. More details on the parts and the assembly of the rainout-shelters are given in Supplementary Tables 1, 2. A blank-free cutting plan for the pipes, the distances between band holders and details on the adaptor plates for the rain-gutter brackets, the holders for the acrylic glass bands and the clamping claws are shown in Supplementary Figures 7–12. One rainout-shelter as we present it in this study costs 730€ (630€ for a control shelter).

Data Collection

To assess the actual percentage of precipitation intercepted by the rainout-shelters, we used the precipitation data from a close-by weather station in Therwil, Switzerland (http://www.bodenmessnetz.ch/messwerte/datenabfrage) as well as data from the on-field meteorological station (Campbell-CR1000) and regularly measured the amount of intercepted precipitation in the rain barrels. We then subtracted the average amount of precipitation collected in the rain barrels from the amount of rain that fell on the ambient control plot (6.25 m^2) to calculate the actual percentage of precipitation that was intercepted by the rainout-shelters.

From April to June 2017 we took weekly measurements at three randomly chosen locations within the center of all 24 subplots to assess volumetric soil water content in 0–6 cm depth (in approx. 75 cm^3 soil) using a handheld Time Domain Reflectometry (TDR)-device (ML-2x ThetaProbe, Delta-T). Each month, we sampled soil in the center of all experimental plots (0–20 cm depth), oven-dried the soil sample to constant weight, and calculated the soil water content (% water, based on g H_2O/g dry weight). In May 2017, we assessed the extent of lateral water movement ("edge effect") under the rainout-shelter and the rainout-shelter control in a subset of 2 plots, each along transects from north to south and from west to east (see also Yahdjian and Sala, 2002). Along each transect, we measured the volumetric water content using the TDR device in 0–6 cm depth in triplicates at 13 measurement positions (25 cm apart from each other, see Supplementary Figure 2). For each transect, rainout-shelter type and plot, we performed a one-way ANOVA to assess the effect of the measurement position (distance from shelter edge) on the soil water content, followed by a Tukey's honestly significance *post-hoc* test. We confirmed the fit of the models by visual inspection of the residual plots, which did not reveal any obvious deviations from homoscedasticity or normality.

We assessed possible shelter effects on the microclimate using iButtons temperature loggers (DS1922L/T/E/S; accuracy: 0.0625°C, 1 record/h) by constantly measuring air temperature at a height of 0.1 m in the center of the respective subplots (total $N = 3$ subplots, each one iButton in a rainout-shelter treatment, a rainout-shelter control treatment and an ambient control treatment), and 1.2 m (total $N = 6$ subplots, each one iButton per treatment in 2 plots) as well as on soil temperature at 0.1 m depth (total $N = 6$ subplots, each one iButton per treatment, 2 plots). For each of the three temperature datasets, we calculated a daily mean temperature to determine the day with the highest and lowest temperature, respectively. We then averaged the individual hourly temperature readings of the highest temperature day, the respective previous and following day for each of the three treatments to calculate mean differences and standard deviations between rainout-shelter treatments and ambient control plots. We used this information to describe potential shelter artifacts under the two most extreme environmental scenarios. In the same way, we also proceeded with the lowest temperature day. In cases the lowest/highest day was the first/last day of the recording period, we used the two following or preceding days, respectively.

We harvested aboveground biomass of the wheat plants 4, 8, and 13 weeks after rainout-shelter establishment from subplots (20 × 50 cm, 2 wheat rows), each subplot located in the core area of the experimental plots.

The analysis of all data and drawing of all figures (excluding the CAD drawings) were done using R (R Core Team, 2016) and the package *ggplot2* (Wickham, 2009). CAD drawings were created with Siemens NX.

RESULTS

Precipitation Interception, Soil Moisture, and Edge Effect

In total, precipitation under the rainout-shelters was 70.6 mm (19th of April to 06th of June 2017) corresponding to a precipitation reduction of 64.9% as compared to the ambient precipitation (201.1 mm) at the study site. This observed value is almost identical to the expected precipitation exclusion values based on a priori calculations for a shelter with 12 bands (−65.2%, **Equation 1**). In the week prior to rainout-shelter establishment (baseline assessment; T0), all treatment plots had comparable soil water contents [ambient control (C): 29.37 ± 1.07% (Mean ±SD), rainout-shelter control (RC): 28.87 ± 1.21%, rainout-shelter (R): 29.10 ± 1.27%; **Figure 2A**]. There was little precipitation between T0 and the first assessment (T1; 21.2 mm in 36 days, **Figure 2C**). Soil water content under both shelter types therefore differed only slightly from the ambient control plots [R: −4.0 percentage points (pp) ± 1.54 pp, $n = 8$, RC: −1.98 pp ± 1.50 pp, $n = 8$] at T1 (35 days after rainout-shelter establishment). The amount of precipitation increased between T1 and the second assessment (T2; 121.6 mm in 27 days; **Figure 2C**) and we recorded more pronounced differences in the soil water content between the rainout-shelter treatment plots and the ambient control plots (R: −11.06 pp ± 0.71 pp, $n = 8$). In contrast, the soil water content in the rainout-shelter control treatment plots was only weakly lower as compared to the ambient control plot (RC: −2.66 pp ± 1.27 pp, $n = 8$). Between T2 and the third assessment (T3), precipitation was low again (75.6 mm in 35 days; **Figure 2C**), and differences between the two rainout-shelters and ambient control decreased (R: −4.68 pp ± 1.65 pp, RC: −2.24 pp ± 1.39 pp).

Data from weekly soil moisture measurements as determined with the TDR device in the top 6 cm of soil also revealed only minor deviations in soil water content between the rainout-shelter control treatment and the ambient control. The data further confirmed that soil moisture content in the rainout-shelter treatment was considerably lower already 1 month after rainout-shelter establishment as compared to the ambient control treatment (**Figure 2B**). Edge effects on soil moisture were only detectable up to 75 cm under shelter the area (**Figures 3A,B**, Supplementary Figures 3A,B).

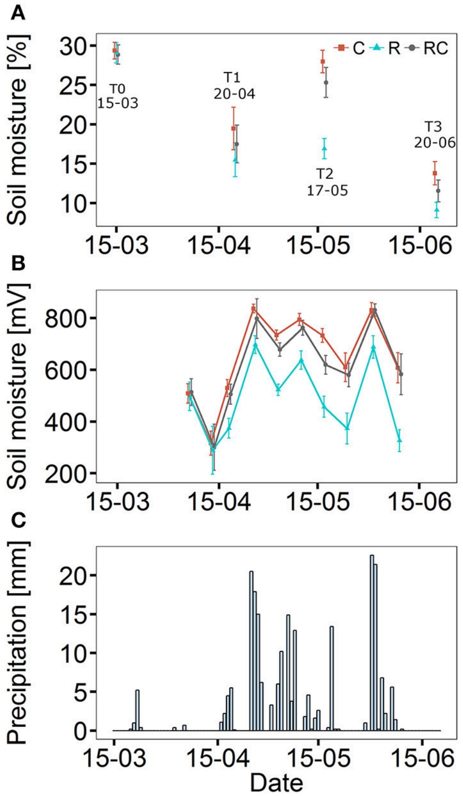

FIGURE 2 | (A) Rainout-shelter effect on soil water content (% water, based on g H_2O/g soil dry weight) as assessed in the top 20 cm (means ± standard deviation, $n = 8$) on March 15, 2017 (baseline assessment; T0), April 20, 2017 (first assessment; T1), May 17, 2017 (second assessment; T2), and June 20, 2017 (third assessment; T3); **(B)** rainout-shelter effects on volumetric soil water content measured with a TDR device (ML-2x ThetaProbe, Delta-T) in 0–6 cm depth. Data points represent means ± standard deviation, $n = 8$; **(C)** Precipitation (mm in 24 h) during the rainfall manipulation experiment. Data between April 5 to May 8, 2017 derived from the online database http://www.bodenmessnetz.ch (station in Therwil), all other data was recorded by the on-site weather station (Campbell-CR1000).

Shelter Effect on Microclimate

Our rainout-shelters had slight impacts on air temperature at 1.2 m height (06th of April to 20th of June 2017; Supplementary Figure 4) in comparison to ambient control plots (R: −0.55 ± 2.76°C, $n = 3648$; RC: −0.59 ± 2.58°C, $n = 3648$). During the period with high ambient temperatures (18th to 20th of June 2017), we recorded reduced temperatures up to 1.0°C in the two rainout-shelter treatments as compared to the ambient control plot (rainout-shelter; R: −0.92 ± 3.46°C, $n = 144$; rainout-shelter control; RC: −0.94 ± 3.3°C, $n = 144$; **Figure 4A**). During the period with rather low temperatures (26th to 28th of April 2017) air temperature was only marginally lower under both rainout-shelter types (R: −0.11 ± 1.27°C, $n = 144$; RC: −0.23 ± 1.28°C, $n = 144$) as compared to the ambient control plots (**Figure 4B**).

The rainout-shelters had very little impact on air temperature at 10 cm above soil surface (07th of April to 05th of June 2017; Supplementary Figure 5) as compared to ambient control plots (R: +0.19 ± 1.25°C, $n = 1,440$; RC: +0.19 ± 1.06°C, $n = 1,440$). Deviations from ambient temperature readings were low during the high (R: +0.11 ± 1.06°C, $n = 72$; RC: +0.19 ± 0.88°C, $n = 72$; **Figure 5A**) and low (R: +0.17 ± 1.56°C, $n = 72$; RC: −0.15 ± 1.36°C, $n = 72$; **Figure 5B**) temperature period.

Similarly, the two rainout-shelter types had little impact on soil temperature (07th of April to 05th of June 2017; Supplementary Figure 6) in comparison to ambient control plots (R: +0.64 ± 0.53°C, $n = 6,076$; RC: +0.39 ± 0.33°C, $n = 6,076$). Deviations from ambient temperature readings were low during the high (R: +1.02 ± 0.46°C, $n = 828$; RC: +0.63 ± 0.31°C, $n = 828$; **Figure 6A**) and low (R: +0.14 ± 0.52°C, $n = 828$; RC: +0.20 ± 0.41°C, $n = 828$; **Figure 6B**) temperature phase.

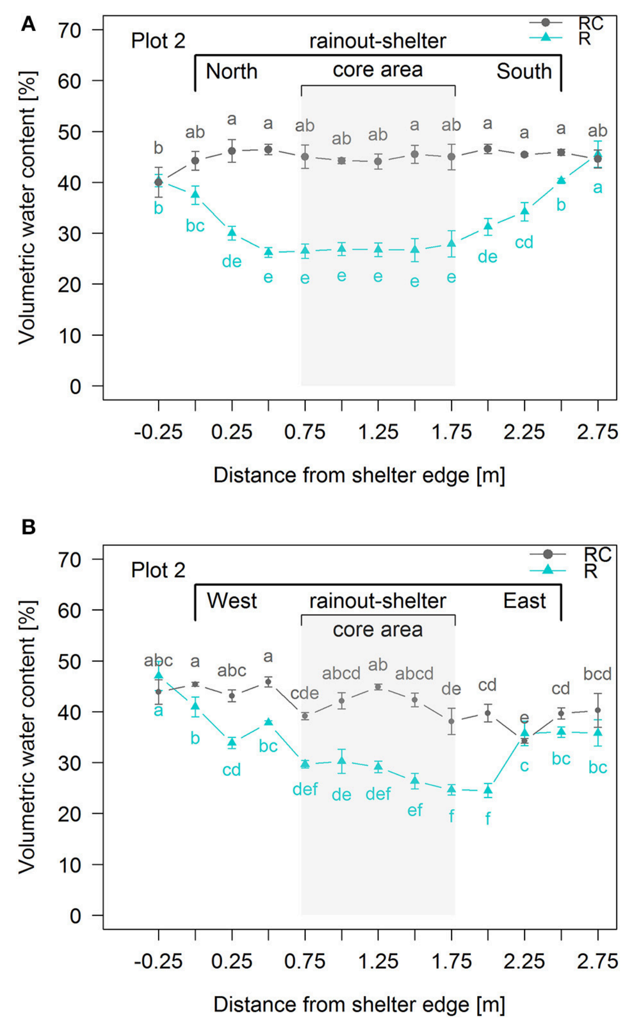

FIGURE 3 | **(A)** Assessment of the soil water content under the rainout-shelter (R) and the rainout-shelter control (RC) using a handheld TDR device (ML-2x ThetaProbe, Delta-T) in the top 6 cm of soil. Data points represent means ± standard deviation, $n = 3$. We measured along transects located **(A)** North–South and **(B)** West-East in two of the eight experimental plots (Plot 2, see also Supplementary Figure 3) on May 15, 2017. Data was analyzed by a one-way ANOVA followed by a Tukey's honestly significance *post-hoc* test. Means within treatments not sharing the same letter are significantly different (Tukey HSD, $P < 0.05$). Rainout-shelters were located with the lower side facing west as this is the prevailing wind direction at the study site.

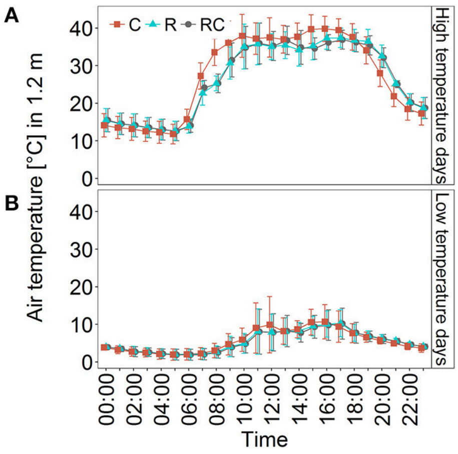

FIGURE 4 | Air temperature (°C) as measured in 1.2 m height using iButton temperature loggers under the rainout-shelter (R), the rainout-shelter control treatment (RC), and the ambient control plots (C). Data points represent hourly temperature measurements (means ± standard deviation) of two plots and 3 days during **(A)** a high temperature phase (June 18–20, 2017) and **(B)** a low temperature phase (April 26–28, 2017).

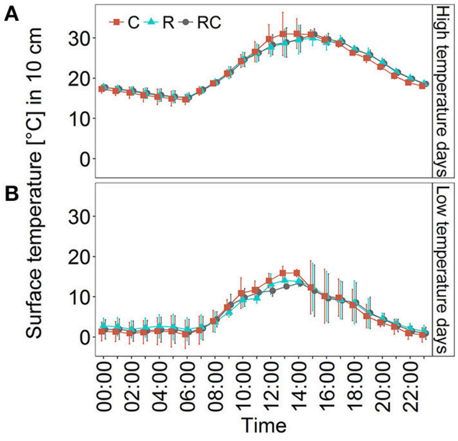

FIGURE 5 | Surface temperature (°C) as measured in 0.1 m height using iButton temperature loggers under the rainout-shelter (R), the rainout-shelter control treatment (RC), and the ambient control plots (C). Data points represent hourly temperature measurements (means ± standard deviation) of one plots and 3 days during **(A)** a high temperature phase (May 28–30, 2017) and **(B)** a low temperature phase (April 18–20, 2017).

Shelter Effect on Shoot Biomass Production

Shoot biomass production was not significantly affected by the rain exclusion treatment, neither 4, 8, or 13 weeks after rainout-shelter establishment (data not shown).

DISCUSSION

Previous designs of rainout-shelters revealed several methodological challenges. Rainout-shelters should allow for a predictable alteration of the precipitation amount, minimize artifacts on microclimatic conditions under the shelter, allow for replication across larger spatial scales and be stable enough to persist under field conditions. The rainout-shelter design described here fulfils all these requirements.

Roof Performance

The rainout-shelters effectively excluded 64.9% of the ambient precipitation, very close to the a priori calculated rain exclusion of 65.2%. A precise prediction of the amount of excluded water depending on the number of acrylic bands in the shelter

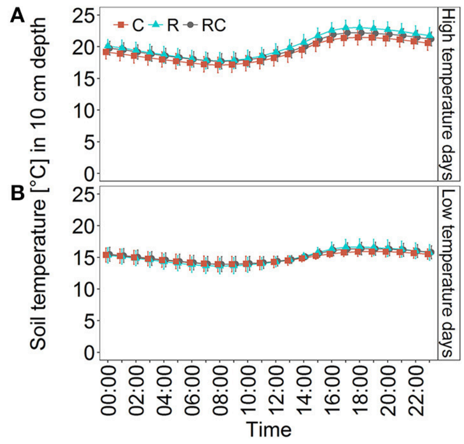

FIGURE 6 | Soil temperature (°C) as measured in 10 cm depth using iButton temperature loggers under the rainout-shelter (R), the rainout-shelter control treatment (RC), and the ambient control plots (C). Data points represent hourly temperature measurements (means ± standard deviation) of two plots and 3 days during **(A)** a high temperature phase (June 18–20, 2017) and **(B)** a low temperature phase (June 6–8, 2017).

construction is thereby possible and provides a crucial tool for the planning of field experiments.

In addition to measurements of rain drainage and natural precipitation levels, soil water content is an important parameter for the evaluation of the performance of rainout-shelters. Soil water content was very similar in the rainout-shelter control and the ambient control treatment during the whole sampling campaign, and lowest in the rainout-shelter treatment for most of the study period. After an initial phase with similar soil water content in each of the three treatments, soil water content was constantly lower under the rainout-shelter as compared to the ambient control and the rainout-shelter control plots. The soil water content in the experimental treatments started to differ after the first heavy rain events supporting results of previous studies (Mikkelsen et al., 2008; Vogel et al., 2013). Although the early summer 2017 was characterized by several short drought-like periods, the developed rainout-shelters still resulted in differences in soil water content, making the design also suitable for regions with drier climatic conditions.

The spatial extent of an edge effect defines the size of the suitable sampling area under a rainout-shelter. However, only few studies determined edge effects by measurements in the field (Yahdjian and Sala, 2002; Beier et al., 2004). In this study, the maximum edge effect was 0.75 m beyond the edges of the 2.5 × 2.5 m roof area, resulting in a 1.00 × 1.00 m core area receiving full treatment effect and thereby being available for measurements. The assessment of edge effects was conducted after a period of rain events, so that the edge effect of 0.75 m can be considered as the maximum edge effect. The chosen dimensions of the rainout-shelters (2.5 × 2.5 × 1.2–1.7 m), which mainly determine the size of the edge effect, result in a reasonable balance between available sampling area, handling and material costs.

The performance of the rainout-shelter material in terms of stability and practicability was excellent. The construction was not damaged by heavy hail storms or rain events as well as temperatures below 0 and above 30°C, and the UV transparent bands did not show any signs of weathering over the study period. The plastic rain gutters slightly deformed during hot summer days and should be replaced by metal rain gutters, especially at field sites with higher maximum temperatures. The specific requirements of field studies in agricultural areas, i.e., the need to remove the shelters for management activities, were successfully met by our removable rainout-shelter construction (note that at least four people are needed to move the rainout-shelter). The workload for maintenance was limited to the drainage of the water barrels which took place every 1 to 2 weeks, depending on precipitation events. This limited workload for maintenance allows managing several replicated rainout-shelters even if in use for longer periods of time.

Microclimate

Rainout-shelters may cause lower air temperature due to the interception of radiation (Yahdjian and Sala, 2002), on the one hand, on the other hand a greenhouse effect, enhanced by reduced air flow under shelters, may cause higher temperature (Svejcar et al., 1999; Vogel et al., 2013). Both artifacts may bias the results of rainout-shelter experiments.

In this study, air temperature at 1.2 and 0.1 m height above ground was little affected by the rainout-shelter, especially during periods of low ambient temperature. This suggests that the spacing of the acrylic bands allowed sufficient airflow to prevent greenhouse effects under the rainout-shelters. A setup with more acrylic bands and subsequently a narrower spacing between bands, however, may have stronger impact on the temperature regimes. The facilitation of air movement is especially crucial in systems with high and dense plant growth such as cereal crops. In the current study, winter wheat plants in their final growth stages almost reached the height of the rainout-shelter, but temperature measurements still did not indicate greenhouse effects (**Figure 4A**). However, differences in air temperatures of up to 1°C during periods of high ambient temperature confirm the need of a rainout-shelter control treatment. Our data showed virtually identical temperature under the two rainout-shelter types (**Figures 4**, **5**, Supplementary Figures 4, 5), thereby supporting the suitability of a rainout-shelter control, especially under constantly warm conditions.

Soil temperature was slightly higher under the rainout-shelter as compared to the ambient control, but only during periods of high ambient temperature and differences to the control plot were more pronounced under the rainout-shelter than under the rainout-shelter control (**Figure 6**). This might be caused by lower soil moisture under the rainout-shelter and consequently lower total water content that buffer heating of the soil by solar radiation. Accordingly, the soil temperature was highest in the rainout-shelter treatment in which soil moisture contents were lowest and only slightly increased in the rainout-shelter control treatment which had intermediate soil moisture contents. This artifact cannot be avoided, because lower soil moisture is the goal of the rainout-shelter.

The interception of radiation was minimized by the use of highly UV-permeable acrylic glass bands as roof material (transmission at 315 nm ≥80, transmission 380–780 nm ≥90%). The use of this material guaranteed natural PAR levels under the rainout shelters.

Plant Performance

The production of above-ground biomass was not significantly affected by the exclusion of rain, neither 4, 8, or 13 weeks after rainout-shelter establishment. We suspect that the exclusion level we selected was not sufficient to dry out the soil within the relatively short duration of our experiment. In order to reduce soil moisture also at lower depths, it seems necessary to extend the duration of the experiment and/or increase the amount of excluded rainfall. It is notable that in annual crop fields longer exclusion periods are almost not possible during the growing season (tillering to harvest is only a few months) and that a more complete exclusion of rainfall over such periods is unrealistic according to all climate change scenarios.

CONCLUSION

The rainout-shelter design presented here is well-suited for experimental manipulations of precipitation in open land ecosystems and agricultural fields in particular. Microclimatic conditions under the rainout-shelter were largely unaffected and the intended alteration of precipitation levels followed our a-priori calculations. Slightly lower under-shelter air temperatures during high ambient temperature phases were the only unintended artifacts we measured. These artifacts were reflected by the rainout-shelter control treatment allowing to account for them. Soil moisture differences between the different treatments established after the first rain events and remained present throughout the experiment. Animated 3-D drawings of the rainout-shelter design (note that the PDF reader needs to be able to show animated PDFs), detailed descriptions of shelter construction, manuals for their setup and a list of material allow future users to apply the developed design in their studies. With this study, the authors hope to promote the use of rainout-shelters to simulate and investigate climate change effects on agricultural systems, which is crucial given the risk of crop yield losses under altered future precipitation regimes.

AUTHOR CONTRIBUTIONS

DK has been involved in the conception of the rainout-shelters and their set-up, was in charge of the field study, has collected and analyzed the data and has written the manuscript. SM has been involved in the rainout-shelter set-up, has collected and analyzed the data and has written the manuscript. HB has designed and constructed all major parts of the rainout-shelters, was in charge of the setup manual, the final decision of materials and suppliers and the CAD drawings. AF has been involved in the conception of the rainout-shelters and the study design, and has commented on the manuscript. PM contributed to the conception of the rainout-shelters, was involved in the development and conductance of the field study in the DOK trial and commented on the manuscript. SS has contributed to the rainout-shelter design and commented on the manuscript. MvK has contributed to the sampling design and statistical analyses, provided the iButtons and commented on the manuscript. KB has developed the idea for rainout-shelter experiments in the framework of this project, has reviewed the existing literature on rainout-shelter experiments and has contributed to design decisions and identification of suitable materials and suppliers. He has contributed to the manuscript.

ACKNOWLEDGMENTS

We thank S. Grau, M. Sauter, F. Perrochet, and J. Meier for their help with field work and M. Tichy at Bröking-Plastic GmbH & Co. KG for the detailed advice on acrylic glass options. We further acknowledge support by the Open Access Publication Funds of the Göttingen University. We also thank the two referees for their constructive input. This research was funded through the 2015–2016 BiodivERsA COFUND call for research proposals, with the national funders Estonian Research Council (ETAG), German Research Foundation (DFG), Ministry of Economy and Competitiveness (MINECO), The Swedish Research Council (Formas), and Swiss National Science Foundation (SNSF).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2018.00014/full#supplementary-material

REFERENCES

Beier, C., Beierkuhnlein, C., Wohlgemuth, T., Penuelas, J., Emmett, B., Körner, C., et al. (2012). Precipitation manipulation experiments – challenges and recommendations for the future. *Ecol. Lett.* 15, 899–911. doi: 10.1111/j.1461-0248.2012.01793.x

Beier, C., Emmett, B., Gundersen, P., Tietema, A., Estiarte, M., Gordon, C., et al. (2004). Novel approaches to study climate change effects on terrestrial ecosystems in the field: drought and passive nighttime warming. *Ecosystems* 7, 583–597. doi: 10.1007/s10021-004-0178-8

Blankinship, J. C., Niklaus, P. A., and Hungate, B. A. (2011). A meta-analysis of responses of soil biota to global change. *Oecologia* 165, 553–565. doi: 10.1007/s00442-011-1909-0

Canarini, A., Carrillo, Y., Mariotte, P., Ingram, L., and Dijkstra, F. A. (2016). Soil microbial community resistance to drought and links to C stabilization in an Australian grassland. *Soil Biol. Biochem.* 103, 171–180. doi: 10.1016/j.soilbio.2016.08.024

Castro, H. F., Classen, A. T., Austin, E. E., Norby, R. J., and Schadt, C. W. (2010). Soil microbial community responses to multiple experimental climate change drivers. *Appl. Environ. Microbiol.* 76, 999–1007. doi: 10.1128/AEM.02874-09

CH2011 (2011). *Swiss Climate Change Scenarios CH2011*. Zurich: C2SM, MeteoSwiss, ETH, NCCR Climate and OcCC.

EEA (2017). *Climate Change, Impacts and Vulnerability in Europe 2016*. Luxembourg: Publications Office of the European Union.

Falloon, P., and Betts, R. (2010). Climate impacts on european agriculture and water management in the context of adaptation and mitigation - the importance of an integrated approach. *Sci. Total Environ.* 408, 5667–5687. doi: 10.1016/j.scitotenv.2009.05.002

Fay, P. A., Carlisle, J. D., Knapp, A. K., Blair, J. M., and Collins, S. L. (2000). Altering rainfall timing and quantity in a mesic grassland ecosystem: design and performance of rainfall manipulation shelters. *Ecosystems* 3, 308–319. doi: 10.1007/s100210000028

Fay, P. A., Carlisle, J. D., Knapp, A. K., Blair, J. M., and Collins, S. L. (2003). Productivity responses to altered rainfall patterns in a C4-dominated grassland. *Ecosyst. Ecol.* 137, 245–251. doi: 10.1007/s00442-003-1331-3

Fliessbach, A., Oberholzer, H. R., Gunst, L., and Mäder, P. (2007). Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. *Agric. Ecosyst. Environ.* 118, 273–284. doi: 10.1016/j.agee.2006.05.022

Gimbel, K. F., Felsmann, K., Baudis, M., Puhlmann, H., Gessler, A., Bruelheide, H., et al. (2015). Drought in forest understory ecosystems – a novel rainfall reduction experiment. *Biogeosciences* 12, 961–975. doi: 10.5194/bg-12-961-2015

IPCC (2014). *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of theIntergovernmental Panel on Climate Change*. Geneva: IPCC.

Mäder, P., Fliessbach, A., Dubois, D., Gunst, L., Fried, P., and Niggli, U. (2002). Soil fertility and biodiversity in organic farming. *Science* 296, 1694–1697. doi: 10.1126/science.1071148

Mikkelsen, T. N., Beier, C., Jonasson, S., Holmstrup, M., Schmidt, I. K., Pilegaard, K., et al. (2008). Experimental design of multifactor climate change experiments with elevated CO_2, warming and drought: the CLIMAITE project. *Funct. Ecol.* 22, 185–195. doi: 10.1111/j.1365-2435.2007.01362.x

Olesen, J. E., and Bindi, M. (2002). consequences of climate change for european agricultural productivity, land use and policy. *Eur. J. Agron.* 16, 239–262. doi: 10.1016/S1161-0301(02)00004-7

Parra, A., Ramírez, D. A., Resco, V., Velasco, Á., and Moreno, J. M. (2012). Modifying rainfall patterns in a mediterranean shrubland: system design, plant responses and experimental burning. *Int. J. Biometeorol.* 56, 1033–1043. doi: 10.1007/s00484-011-0517-3

Poll, C., Marhan, S., Back, F., Niklaus, P. A., and Kandeler, E. (2013). Field-scale manipulation of soil temperature and precipitation change soil CO_2 flux in a temperate agricultural ecosystem. *Agric. Ecosyst. Environ.* 165, 88–97. doi: 10.1016/j.agee.2012.12.012

Porporato, A., Daly, E., and Rodriguez-Iturbe, I. (2004). Soil water balance and ecosystem response to climate change. *Am. Nat.* 164, 625–632. doi: 10.2307/3473173

Prechsl, U. E., Burri, S., Gilgen, A. K., Kahmen, A., and Buchmann, N. (2015). No shift to a deeper water uptake depth in response to summer drought of two lowland and sub-alpine C_3-grasslands in Switzerland. *Oecologia* 177, 97–111. doi: 10.1007/s00442-014-3092-6

R Core Team (2016). *R: A Language and Environment for Statistical Computing*. Vienna. Available online at: https://www.r-project.org/

Russo, S., Dosio, A., Sterl, A., Barbosa, P., and Vogt, J. (2013). Projection of occurrence of extreme dry-wet years and seasons in Europe with stationary and nonstationary standardized precipitation indices. *J. Geophys. Res. Atmos.* 118, 7628–7639. doi: 10.1002/jgrd.50571

Spinoni, J., Naumann, G., and Vogt, J. (2015). Spatial patterns of European droughts under a moderate emission scenario. *Adv. Sci. Res.* 12, 179–186. doi: 10.5194/asr-12-179-2015

Svejcar, T., Angell, R., and Miller, R. (1999). Fixed location rain shelters for studying precipitation effects on rangelands. *J. Arid Environ.* 42, 187–193.

Trnka, M., Oelsen, J. E., Kersebaum, K. C., Skjelvag, A. O., Eitzinger, J., Seguin, B., et al. (2011). Agroclimatic conditions in europe under climate change. *Glob. Chang. Biol.* 17, 2298–2318. doi: 10.1111/j.1365-2486.2011.02396.x

Vogel, A., Fester, T., Eisenhauer, N., Scherer-Lorenzen, M., Schmid, B., Weisser, W. W., et al. (2013). Separating drought effects from roof artifacts on ecosystem processes in a grassland drought experiment. *PLoS ONE* 8:e70997. doi: 10.1371/journal.pone.0070997

Wickham, H. (2009). *ggplot2: Elegant Graphics for Data Analysis*. New York, NY: Springer-Verlag.

Wu, Z., Dijkstra, P., Koch, G. W., Penuelas, J., and Hungate, B. A. (2011). Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. *Glob. Chang. Biol.* 17, 927–942. doi: 10.1111/j.1365-2486.2010.02302.x

Yahdjian, L., and Sala, O. E. (2002). A rainout shelter design for intercepting different amounts of rainfall. *Oecologia* 133, 95–101. doi: 10.1007/s00442-002-1024-3

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