PLANT COMPETITION IN A CHANGING WORLD

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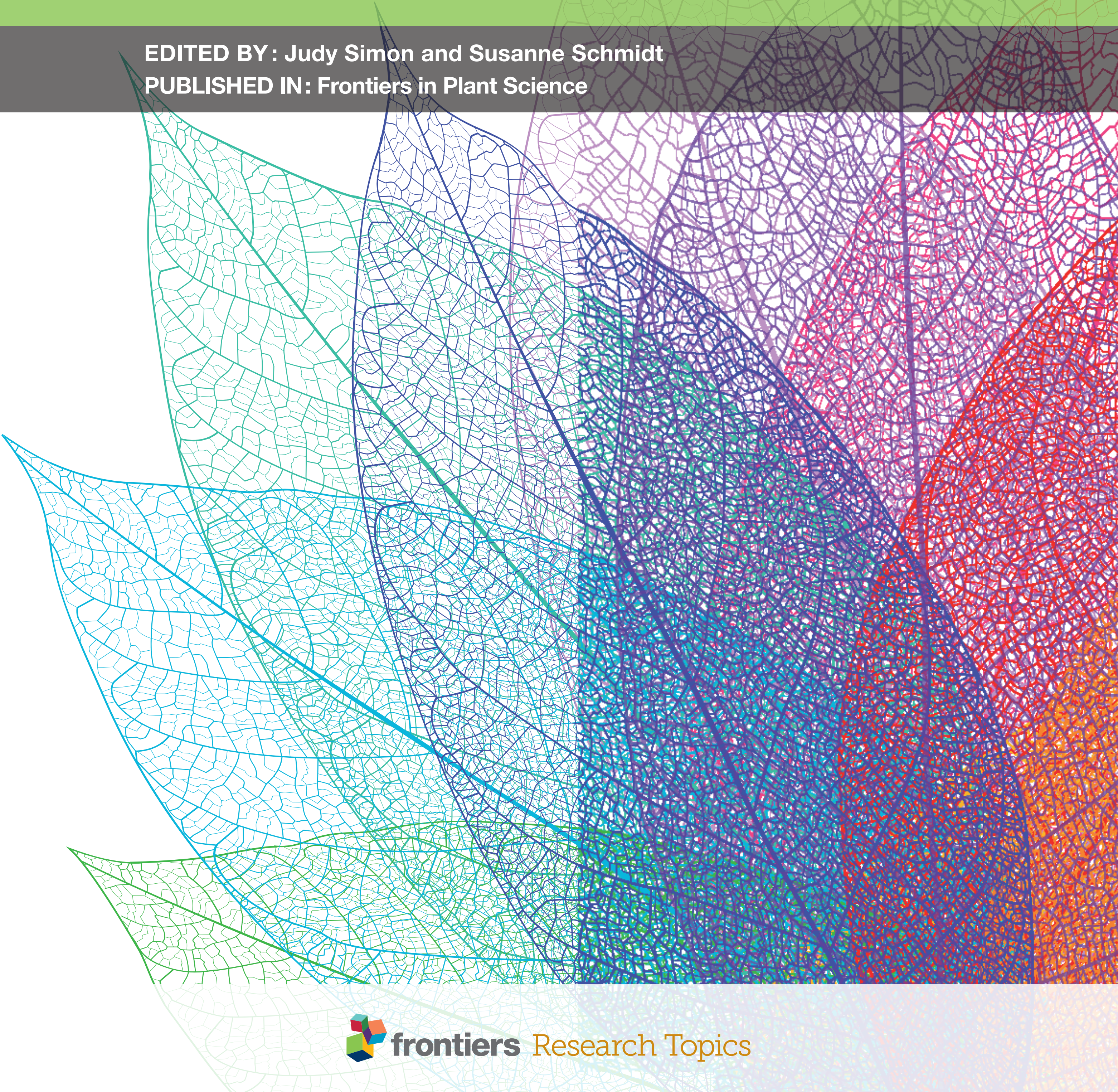

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PLANT COMPETITION IN A CHANGING WORLD

Topic Editors:
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Competitiveness describes a key ability important for plants to grow and survive abiotic and biotic stresses. Under optimal, but particularly under non-optimal conditions, plants compete for resources including nutrients, light, water, space, pollinators and other. Competition occurs above- and belowground. In resource-poor habitats, competition is generally considered to be more pronounced than in resource-rich habitats. Although competition occurs between different players within an ecosystem such as between plants and soil microorganisms, our topic focusses on plant-plant interactions and includes inter-specific competition between different species of similar and different life forms and intra-specific competition.

Strategies for securing resources via spatial or temporal separation and different resource needs generally reduce competition. Increasingly important is the effect of invasive plants and subsequent decline in biodiversity and ecosystem function. Current knowledge and future climate predictions suggest that in some situations competition will be intensified with occurrence of increased abiotic (e.g. water and nutrient limitations) and biotic stresses (e.g. mass outbreak of insects), but competition might also decrease in situations where plant productivity and survival declines (e.g. habitats with degraded soils).

Changing interactions, climate change and biological invasions place new challenges on ecosystems. Understanding processes and mechanisms that underlie the interactions between plants and environmental factors will aid predictions and intervention. There is much need to develop strategies to secure ecosystem services via primary productivity and to prevent the continued loss of biodiversity.

This Research Topic provides an up-to-date account of knowledge on plant-plant interactions with a focus on identifying the mechanisms underpinning competitive ability. The Research Topic aims to showcase knowledge that links ecological relevance with physiological processes to better understanding plant and ecosystem function.

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EDITORIAL
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Editorial: Plant Competition in a Changing World

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Keywords: competition, climate change, invasion, conservation, allelochemicals, global warming, facilitation, plant–plant interactions

Editorial on the Research Topic

Plant Competition in a Changing World

Climate change and biological invasions place new challenges on plants, their development, fitness, and competitiveness. To develop and evaluate strategies for sustainable ecosystem management and to respond to biodiversity loss, we need mechanistic understanding of the changes that are occurring in plant communities. Underlying drivers of change are plant–plant interactions which include competition, facilitation, and avoidance of competition, and their regulation by environmental factors (Trinder et al., 2013). The studies highlighted in this ebook examine plant competition in range of communities that span from forests to meadow and crop systems across alpine, temperate, and tropical climates.

Facilitation, positive interactions between plant species, is a key driver of plant community dynamics and structure, but comparatively few studies have examined how facilitation is modulated in response to climate change (Brooker, 2006; Brooker et al., 2007; Lavergne et al., 2010). In their review, Anthelme et al. discuss four aspects of facilitative effects in alpine systems in response to climate change: (1) a reduction of facilitative effects in alpine plant presence in response to declining cold-temperature stress due to warming in established alpine systems, (2) an increase in facilitative effects as a response to migration to colder environments with higher elevation, (3) changing patterns of facilitation along latitudinal gradients, and (4) the potential of nurse plants to buffer changes in microhabitats. Anthelme et al. present different migration scenarios that include various types of facilitation in response to increasing temperature. Valladares et al. review the consequences of facilitation and competition in context of global change, encompassing climate change, and biological invasions, with a focus on phylogenetic relatedness, functional traits, and phenotypic plasticity. Valladares et al. summarize the direct and indirect drivers of species richness in different ecosystems, such as temperate and tropical forests, grasslands, and alpine systems. The authors argue that studying pauci-specific communities will provide the necessary understanding on species interactions in more complex systems. The reviews by Valladares et al. and Anthelme et al. conclude that although there is no doubt that climate change impacts on plant communities directly (e.g., via increasing temperatures) and indirectly (e.g., via changes in the interactions between species), further empirical knowledge is needed to advance understanding of the underlying mechanisms of plant–plant interactions in different plant communities, climate, and resource settings. For example, studies on alpine systems are biased toward certain regions, especially Europe, with other regions overlooked. The authors recommend examining systems in which single species and their intra-specific functional variability are important to expand from the current focus on species-rich systems such as tropical rainforests.

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The other key driver discussed here is **competition**, especially in resource-poor habitats where plant growth and reproduction is challenging and/or further impaired with global change. A plant's ability to occupy space influences its ability to access resources such as light, water, and nutrients. Studying the relative influence of topography, environment, and spatial distance of rockcress (*Boechera stricta*) to other individuals, either intra- or inter-specific, Naithani et al. found that this species' performance in a meadow community is predominantly influenced by intra-specific competition and insect herbivory. In contrast, its spatial distribution in the meadow community is limited by dispersal and microhabitat preference. At the other end of the plant size spectrum and focusing on competition for light, van Kuij et al. present a 3D-model, validated with field data, for calculating photosynthesis rates for individual trees in forests. Such model has potential to assist forest management strategies, such as aiding the potential effect of accelerated succession to generate resilient forests/plantations.

Prior and Bowman investigated the interaction between tree growth and microhabitat across a macro-ecological gradient. They present new evidence from an extensive dataset of eucalypt tree growth collected across temperate and sub-tropical mesic Australia that in cooler habitats with sufficient water availability, light is the most limiting resource which results in increased competition, whereas in hot and dry habitats where water is the limiting factor, light is no longer driving competition. The study by Muller et al. on species interactions in urban plantings at three buildings in subtropical Australia, expands on the relationships of light and water, and demonstrates that plant productivity and arthropod diversity increase in situations with abundant availability of resources. This study provides evidence that ecological principles are transferrable from natural systems to human-made urban systems. Expanding on plant–plant competition from light and water, the study by Li et al. examines nitrogen as a main macronutrient that limits plant growth in many plant communities, and demonstrate that competition is reduced in two co-occurring tree species, beech and sycamore maple, that have a preference for organic and inorganic nitrogen forms, respectively. Another mechanism to avoid competition is allelopathy, the release of plant-growth inhibiting or toxic substances into the rhizosphere. Asaduzzaman et al. identified potentially allelopathic compounds in a laboratory bioassay investigating root and shoot tissue of different canola cultivars when growing in competition with weeds. The authors suggest that an allelopathic effect depends not only on the synthesis of certain compounds, but also on their active exudation into the rhizosphere and this seems to be dependent on intrinsic genotypic factors.

Plant invasions and their contribution to the competition for resources in native plant communities were reviewed by Gioria and Osborne. The authors discuss how "winning" the competition depends on factors that include resource distribution and stage of the invasion process, and that raise conceptual and methodological issues for future studies on competition in plant invasions. Considering environmental, such as competition for nutrients, water, light, and space, as well as biotic constraints, they find "windows of opportunity" during which competition is reduced. Furthermore, Gioria and Osborne show seasonal shifts between environmental or biotic constraints as key drivers of competition. Plant invasions and their role in plant–plant competition for resources has also been the focus of several original research articles here. Elgar et al. showed facilitation can provide a measure to overcome competition between native woody plants and invasive grasses in rainforest reforestation on former agricultural land. Ens et al. used leaf-scale ecophysiological and stand-scale growth traits between an invasive and a native grass and present evidence that the higher photosynthetic nitrogen use-efficiency of the invasive grass selects for improved nitrogen acquisition from soil in nitrogen-poor ecosystem. Exploring the foraging responses and performance of herbaceous invaders to nitrogen-rich patches, Keser et al. suggest that strong plasticity of root-foraging responses is adaptive and appears to contribute to invasiveness.

Overall, plant–plant competition and facilitation present a framework for understanding changes in plant communities. Such interactions are likely to become more prevalent where plants have to increasingly secure resources in response to climate change. Current knowledge together with climate predictions indicate that in some habitats competition will intensify with increased abiotic stress (e.g., water and nutrient limitations). Adding biotic stresses, such as plant invasions, will further impact on native plant communities with outcomes including declining biodiversity and ecosystem function. To date, different empirical approaches have mainly been used separately; however, using them in combination would increase resolution (Valladares et al.). Including multiple potential drivers of plant interactions in combinations in future studies, would aid in developing and evaluating strategies for sustainable ecosystem management to secure ecosystem services for modern society.

AUTHOR CONTRIBUTIONS

JS wrote the first draft of the editorial, both JS and SS then jointly edited the final version.

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Facilitation among plants in alpine environments in the face of climate change

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While there is a large consensus that plant–plant interactions are a crucial component of the response of plant communities to the effects of climate change, available data remain scarce, particularly in alpine systems. This represents an important obstacle to making consistent predictions about the future of plant communities. Here, we review current knowledge on the effects of climate change on facilitation among alpine plant communities and propose directions for future research. In established alpine communities, while warming seemingly generates a net facilitation release, earlier snowmelt may increase facilitation. Some nurse plants are able to buffer microenvironmental changes in the long term and may ensure the persistence of other alpine plants through local migration events. For communities migrating to higher elevations, facilitation should play an important role in their reorganization because of the harsher environmental conditions. In particular, the absence of efficient nurse plants might slow down upward migration, possibly generating chains of extinction. Facilitation–climate change relationships are expected to shift along latitudinal gradients because (1) the magnitude of warming is predicted to vary along these gradients, and (2) alpine environments are significantly different at low vs. high latitudes. Data on these expected patterns are preliminary and thus need to be tested with further studies on facilitation among plants in alpine environments that have thus far not been considered. From a methodological standpoint, future studies will benefit from the spatial representation of the microclimatic environment of plants to predict their response to climate change. Moreover, the acquisition of long-term data on the dynamics of plant–plant interactions, either through permanent plots or chronosequences of glacial recession, may represent powerful approaches to clarify the relationship between plant interactions and climate change.

Keywords: competition, cushion plants, early snowmelt, facilitation, latitudinal gradient, nurse plant, stress-gradient hypothesis, global warming

INTRODUCTION

Empirical studies and reviews in the last decade leave no doubt that the multiple effects of current and predicted climate change will affect plant communities not only directly, e.g., via "thermophilization," but also indirectly, through changes in interactions among species (Lortie et al., 2004; Brooker, 2006; Poloczanska et al., 2008; Gilman et al., 2010; Adler et al., 2012; Gottfried et al., 2012; Grassein et al., 2014). To date, however, non-trophic interactions are still poorly considered in predictive models of plant community responses to climate change (Lavergne et al., 2010; Walther, 2010; Bellard et al., 2012; but see Sutherst et al., 2007).

Most efforts aiming at including interactions in predictions of the impact of climate change on plant communities have been based on negative interactions (hereafter termed "competition"; Brooker, 2006; Araújo and Luoto, 2007; Tylianakis et al., 2008; Meier et al., 2012). In comparison, the role of non-trophic positive interactions among species (hereafter termed "facilitation") in driving the structure and dynamics of plant communities under rapid climate change has rarely been tested, even though conceptual models and reviews predict that this type of interaction might be pivotal, especially in harsh environments (Brooker, 2006; Brooker et al., 2007; Lavergne et al., 2010). Improving our knowledge on facilitation among plants under a changing climate is therefore urgently required as part of assessing the impacts of climate change on plant communities and ecosystems.

The stress-gradient hypothesis (SGH) is one of the most important conceptual advances made over the past two decades with respect to plant–plant interactions along environmental gradients. In its current definition, the SGH predicts that positive interactions among plants (and also among animals: Kawai and Tokeshi, 2007; Dangles et al., 2013) will increase with stress and disturbance, at least up to a certain threshold (Bertness and Callaway, 1994; Brooker and Callaghan, 1998; Michalet et al., 2006; Maestre et al., 2009; He et al., 2013; He and Bertness, 2014). Therefore, a central goal in predicting the response of plant communities in a world affected by climate change is to determine to what extent future environments will modify the levels of disturbance and/or stress experienced by plants (*sensu* Grime, 1977). The direct effects of climate change on plants include warmer temperatures, changes in water availability, and a higher occurrence of extreme events such as drought or heat waves (IPCC, 2013). While limitations related to temperature and water may represent stressors for plants, extreme events are related more to disturbance. Importantly, the effect of a given stress or disturbance level on plants is likely to be site- and species-specific. For example, in dry, warm environments such as the Sahara, warming will certainly increase the environmental stress on plants by increasing evapotranspiration (e.g., Johnson et al., 2012), hence decreasing the water availability (McCluney et al., 2012). Conversely, warming in alpine environments is expected to reduce the stress experienced by plants, thus potentially increasing plant productivity (e.g., Cavieres and Sierra-Almeida, 2012). However, the sum of different stresses or disturbances along environmental gradients will not necessarily generate more facilitation, as demonstrated by the possible existence of non-additive models of interactions (Malkinson and Tielbörger, 2010).

Alpine regions represent an important model for examining the effects of climate change on plant–plant interactions for several reasons. First, alpine ecosystems are relatively homogenous in terms of climatic conditions, and they are found on all continents at almost all latitudes, from 0 to 6000 m a.s.l. (Körner, 2003; Nagy and Grabherr, 2009). Alpine plant communities have been widely used by ecologists over the last two decades to infer patterns and mechanisms of plant–plant interactions, in particular because mountain environments provide abrupt stress variations along elevation gradients (Körner, 2007). Studies conducted in these environments have provided major contributions to the definition and further refinements of the SGH (Choler et al., 2001; Callaway et al., 2002; Maestre et al., 2009; He et al., 2013). In most alpine environments, greater facilitation is observed at higher elevation, i.e., in more stressful conditions—readers are referred to the specific cases in dry alpine environments reported by Cavieres et al. (2006) and Michalet et al. (2014) where two opposing stress gradients were found. Accordingly, these environments constitute a sound model for inferring the effects of climate change on the outcomes of plant–plant interactions.

In this paper, we provide an overview of the extent to which facilitation among plants will interact with the effects of climate change in the organization of alpine plant communities in future decades. We provide a review of current knowledge, and suggest directions for future research. In particular, we focus our contribution on the following four issues and associated hypotheses:

(1) *Facilitation in established alpine communities*. Our underlying hypothesis is that decreasing abiotic stress through warming reduces the frequency of facilitative effects among plants that are already established in alpine ecosystems.
(2) *Facilitation in alpine communities migrating to higher elevations*. We hypothesize that alpine plants migrating to higher elevations as a response to the effects of warming will interact more positively because of the harsher environmental conditions in the newly settled areas (primary succession).
(3) *Facilitation along latitudinal gradients.* We hypothesize that the outcomes of plant–plant interactions will change along large latitudinal gradients, together with the magnitude of climate change and characteristics of the alpine environment.
(4) *Facilitation and the long-term buffering effect of alpine nurse plants*. Our hypothesis here is that the persistent buffering effects on microenvironmental conditions by some alpine nurse plants may offer long-term biotic refuges for other alpine plants.

FACILITATION IN ESTABLISHED ALPINE COMMUNITIES: A BIBLIOGRAPHIC REVIEW

On 3 April 2014 we conducted a search of the peer-reviewed literature via Web of Science using the following terms: "plants" AND ("alpine" OR "arctic") AND "climate change" AND ("facilitation" OR "positive interaction"). We obtained a total of 80 references. We then extended this selection by reviewing the relevant literature cited in each of these 80 papers and obtained a second list of 96 references. Later, we reduced this list by retaining only those references that (1) provided explicit data on both climate change and facilitation among plants, and (2) considered facilitation above the treeline (thus excluding forests, but taking into account small and dwarf shrubs). Studies along elevational gradients were only considered if they focussed explicitly on climate change effects. Studies that explicitly documented plant–lichen interactions in the face of climate change were also included. This search resulted in a shortlist of only 17 papers, published between 1997 and 2014 (**Table 1**). To analyse the data, we considered five parameters: the geographical location of studies; the type of climate change effect (warming, snowmelt timing, water availability); the methodology (experimental, observational, modeling); the number of interacting plants (we assumed that studies involving up to four beneficiary species were "species-pair" studies, in contrast to studies at the community level); and the net interaction outcome (more or less facilitation, neutral, or complex effects with no clear trend). We also took into account the effects of three covariables: nutrients, land abandonment, and wind speed. This quantitative method was not used for Sections Facilitation and the Upward Migration of Alpine Species, Facilitation and Climate Change Along Latitudinal Gradients, and Long-term Facilitative Effects by Nurse plants because of the scarcity of available literature.

VARIOUS CLIMATE CHANGES EFFECTS, VARIOUS INTERACTION OUTCOMES

The majority of studies (88%; **Table 1**) used temperature warming as a proxy for climate change. Indeed, it is one of the most—if not the most—predictable effects of climate change on alpine environments, either in terms of maximum, minimum,

Table 1 | Review of studies examining facilitation among plants in established alpine communities in the face of climate change.

References	Country/ State	Environment	Effect	Covariable	Protocol	Climate change proxy	Interaction	Neighbor	Target species	Net interaction
Almeida et al., 2013	Ecuador	Tropical alpine	Warming		Observational	Elevation gradient	Species pairs	*Azorella aretioides*	*Lasiocephalus ovatus*	Facilitation decrease
Brooker et al., 2007	Global	Global	Warming		Modeling	Spatial model	Community scale	Mutualists and competitors	Mutualists and competitors	Complex
Cavieres and Sierra-Almeida, 2012	Chile	Dry alpine	Warming		Experimental	OTC	Species pairs	*Azorella madreporica*	*Hordeum comosum*	Facilitation decrease
Cornelissen et al., 2001	Global	Arctic	Warming	Nutrient	Experimental/ Observational	Various	Community scale	Vascular plants	Lichens	Facilitation decrease
Crabtree and Ellis, 2010	Scotland	Alpine	Warming	Wind	Observational	Elevation gradient	Community scale	Vegetation structure	11 lichen species	Complex
Dormann et al., 2004	Norway	Arctic	Warming	Nutrient	Experimental	OTC	Species pairs	*Luzula confusa*, *Salix polaris*	*Luzula confusa*, *Salix polaris*	Facilitation decrease
Hobbie et al., 1999	USA/Alaska	Arctic	Warming		Experimental	OTC	Community scale	Seven species	Community	Neutral
Hülber et al., 2011	Austria	Subalpine	Snowmelt timing		Observational	Snowbed-grassland ecotone	Species pairs	Snowbed community	Four snowbed species	Complex
Klanderud and Totland, 2005	Norway	Alpine	Warming	Nutrient	Experimental	OTC	Species pairs	Vegetation structure	*Thalictrum alpinum, Carex vaginata*	Complex
Klanderud and Totland, 2007	Norway	Alpine	Warming		Experimental	OTC	Community scale	*Dryas octopetala*	Seeds of 27 alpine species	Facilitation decrease
Klanderud, 2005	Norway	Alpine	Warming	Nutrient	Experimental	OTC	Species pairs	*Dryas octopetala*	*Thalictrum alpinum, Carex vaginata*	Facilitation decrease
Michalet et al., 2014	Global	Alpine	Warming, water		Observational	Elevation gradient	Community scale	Various	Various	Complex
Olsen and Klanderud, 2014	Norway	Alpine	Warming		Experimental	OTC	Community scale	*Dryas octopetala*	Seeds of 27 alpine species	Facilitation decrease
Pajunen et al., 2011	Finland, Russia	Arctic	Warming		Observational	Shrub abundance	Community scale	Shrubs	Plant community (functional groups)	Complex
Shevtsova et al., 1997	Finland	Subarctic	Warming, water		Experimental	OTC, water addition	Species pairs	*Empetrum nigrum, Vaccinium vitis-idae*	*Empetrum nigrum, Vaccinium vitis-idae*	Complex

(Continued)

Table 1 | Continued

References	Country/ State	Environment	Effect	Covariable	Protocol	Climate change proxy	Interaction	Neighbor	Target species	Net interaction
Vittoz et al., 2009	Switzerland	Subalpine	Warming	Land abandonment	Observational	Permanent plots	Community scale	Community	Community	Facilitation decrease
Wipf et al., 2006	USA/Alaska	Subarctic	Snowmelt timing		Experimental	Snow manipulation	Species pairs	*Empetrum nigrum, Vaccinium vitis-idae*	*Empetrum nigrum, Vaccinium vitis-idae*	Facilitation increase

or average values (IPCC, 2013). In the majority of these studies (53% of the warming studies), warming decreased the net effects of facilitation among alpine plants, and they never generated an increase in facilitation. In alpine environments, cold temperatures—especially low extremes—are one of the main physical stresses experienced by plants, despite the fact they are generally well adapted to such conditions (Körner, 2003). The buffering of extreme temperature has been shown to be one of the main mechanisms by which nurse plants facilitate other species in alpine regions (Nyakatya and McGeoch, 2008; Badano and Marquet, 2009), supporting the hypothesis that facilitation in alpine environments is primarily generated by stresses that are not directly related to resource availability (Maestre et al., 2009). Therefore, the reduction of temperature stress alongside global warming is expected to reduce the positive effects of alpine nurse plants, lowering net facilitation among plants in established communities, as predicted by the SGH. Facilitation release is thought to be related to (1) higher abundance/cover of competing species (Cornelissen et al., 2001; Vittoz et al., 2009), and (2) a higher growth rate at the individual scale (taller individuals; Klanderud, 2008; Pajunen et al., 2011).

However, the causal relationship between warming and reduced net facilitation among alpine plants is not clear-cut. In one study, reduced facilitation appeared to partly result from land abandonment, which affected plant cover dynamics (Vittoz et al., 2009); whereas, in another study, net facilitation release was possibly compensated by a reduction in wind speed (Crabtree and Ellis, 2010). Five other studies have revealed complex interaction patterns related to (1) site-specific effects (dry vs. temperate; Michalet et al., 2014), (2) species-specific effects (Shevtsova et al., 1997; Klanderud and Totland, 2005; Brooker et al., 2007; Pajunen et al., 2011), and (3) variation in the performance variable used (Klanderud and Totland, 2005). Taken together, these studies suggest uncertainty remains in terms of to what extent warming will reduce the importance of facilitation in the organization of established alpine plant communities. Facilitation release may be more obvious when taking into account interactions between established alpine species and species migrating from lower vegetation belts, as supported extensively in the literature (e.g., Grabherr et al., 1994; Pauli et al., 2012; Olsen and Klanderud, 2014).

Greater nutrient availability, in particular through nitrogen deposition, is expected to be another covariable of climate change in alpine regions (Bobbink et al., 1998; Hu et al., 2014) with well-known positive effects on plant productivity (Alatalo and Little, 2014; McDonnell et al., 2014). Interestingly, four studies (Cornelissen et al., 2001; Dormann et al., 2004; Klanderud, 2005; Klanderud and Totland, 2005) found that climate change may interact with changes in nutrient levels, leading to reduced net facilitation among plants (**Figure 1**). Therefore, it is possible that nutrient enrichment related to nitrogen deposition may be a "hidden" driver of facilitation release in alpine communities also experiencing climate change. This hypothesis is coherent with recent data pointing toward a positive relationship between facilitation and nutrient stress in alpine regions (Yang et al., 2010; Anthelme et al., 2012). These results, supported by a study that took into account nitrogen addition but not warming in the Rocky Mountains (Suding et al., 2008),

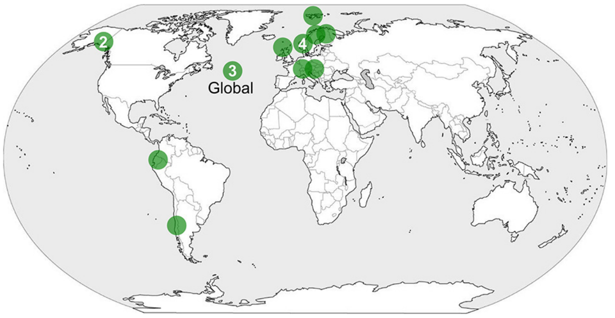

FIGURE 1 | Location of studies documenting facilitation among plants in established alpine communities under the effects of climate change.

may question the assumption of Maestre et al. (2009) that plant interactions in alpine regions are not driven by limitations in resources. Nevertheless, the four nutrient enrichment experiments in our review did not specifically simulate nitrogen deposition, but instead used a more complex mixture of nutrients (e.g., *NPK* in Klanderud, 2005; *Hoagland solution* in Dormann et al., 2004). Accordingly, determining the effects of nitrogen fertilization on plant–plant interactions as a consequence of atmospheric contamination remains a challenge.

FACILITATION AND EARLY SNOWMELT, AN INDIRECT EFFECT OF WARMING

In temperate-alpine and arctic-alpine regions, snow lasts for several months—except in Equator-facing slopes—such that snowmelt timing has been recognized as a central driver of alpine plant distribution (Körner, 2003; Wipf and Rixen, 2010). An increasing number of empirical studies and reviews have shown that (1) warming diminishes the duration of snow cover, and (2) this reduction is responsible for major changes in the cover, diversity, and productivity of alpine plant communities (Schöb et al., 2009; Wipf and Rixen, 2010; Myers-Smith et al., 2011). Only two studies from our review focussed on the effect of snowmelt timing on plant–plant interactions. By examining survival, phenology, growth and reproduction of two alpine shrubs, Wipf et al. (2006) observed more facilitation between two alpine shrubs with earlier snowmelt. Unlike temperature increase and nutrient enrichment, early snowmelt can be considered as an additional stress as it exposes plants to late freezing events (e.g., Wheeler et al., 2014). Thus, in line with the SGH, facilitative interactions are expected to be more frequent with earlier snowmelt, which was sustained by the data of Wipf et al. (2006). However, this experimental study was carried out over only 1 year. It should therefore be questioned whether or not, under the pervasive effects of warming in the longer term, the frequency of late freezing events (spring frosts) at certain sites will decrease, thereby further conditioning the outcome of interactions (Lavergne et al., 2010). The second study in our review adds complexity to this relationship because it showed that plant–plant interactions were not only influenced by snowmelt timing, but also depended on the performance metrics considered (germination vs. survival of seedlings; Hülber et al., 2011).

FUTURE RESEARCH ON ESTABLISHED ALPINE COMMUNITIES

Existing data only partially corroborates our first hypothesis that climate change will decrease the frequency of facilitation among alpine/arctic plants. Indeed, the negative effects of warming on observed facilitation may also be the result of co-occurring parameters such as herbivory or wind speed. Moreover, variation in snowmelt timing might compensate facilitation release. Knowledge on the future of plant–plant interactions in established alpine plant communities under climate change is generally scarce. Without such data, even well-conceived conceptual models taking into account plant–plant interactions (e.g., Brooker et al., 2007) may not be able to predict the future of alpine communities under the effects of climate change (Sutherst et al., 2007). Focussing future research on alpine and arctic regions that have thus far been overlooked, including East and North Africa, Papua New Guinea, New Zealand, the Himalaya, Central Asia, Siberia, the Caucasus, the Rocky Mountains, Patagonia, and Antarctica, will certainly add consistency to the hypotheses raised above. Considering not only warming but also focussing on other predictable effects such as snowmelt timing and other environmental changes (herbivory, atmospheric nutrient deposition) is required. Given the fact that extreme events related to temperature or precipitation are expected to occur at a higher frequency in the future, and change the outcomes of plant–plant interactions (e.g., Saccone et al., 2009), the absence of studies taking this factor into account in alpine regions creates bias and requires further research (Wipf et al., 2013). The most challenging climate change effect to study is precipitation, which is difficult to predict given the interplay between global and local factors (Murphy et al., 2004; Loarie et al., 2009).

Another challenging avenue of research for the future is to quantify the cost of being an alpine nurse plant under the effects of climate change. A recent global study in alpine regions evidenced that increasing cover of beneficiary species limited the reproductive output of associated nurse plants (Schöb et al., 2014b), suggesting that a possible increase in the growth rate of alpine beneficiary species because of warming (e.g., Pajunen et al., 2011) may have a negative impact on nurse plants, eventually being a possible cause of population extinction. However, another study observed a reduced negative effect of beneficiary species on nurse plants in more productive ecosystems (Schöb et al., 2014a), possibly indicating that in warmer, more productive environments, negative feedback effects of beneficiary species on nurse plants might diminish. From these seemingly contradictory viewpoints, understanding the feedback effects of beneficiary species on nurse plants in the face of climate change constitutes an important and challenging topic for the future.

From a methodological standpoint, the relatively high proportion of studies on interactions conducted at the community level (53%; **Table 1**) is encouraging, because the pairwise approach may not provide a representative view of the overall patterns of plant–plant interactions (*species-specific effects*: Cavieres and Badano, 2009; Soliveres and Maestre, 2014). Moreover, the development over the past two decades of open-top chambers (OTC) for experimentally manipulating temperature in alpine-arctic environments has been a crucial development for the study of alpine plant communities (protocol ITEX: Marion et al., 1997;

Figure 2). Its use for predicting plant–plant interactions against a background of warming has provided consistent data in alpine and arctic environments (**Table 1**: 8 studies). It provides a necessary balance between observational and experimental studies, as found in our review (**Table 1**; Schöb et al., 2012). Other types of manipulative methodological approaches, such as freezing experiments (Martin et al., 2010), CO_2 enrichment (Hättenschwiler et al., 2002), snow cover manipulation (review in Wipf and Rixen, 2010), ozone concentration and associated UV-B radiation (Searles et al., 2001), and extreme events (Jentsch et al., 2007), should help to develop a more precise conceptual framework of plant–plant interactions by the side effects of climate change in alpine regions.

FACILITATION AND THE UPWARD MIGRATION OF ALPINE SPECIES

INCREASED STRESS FOR PLANTS DURING ACCELERATED PRIMARY SUCCESSION

In the previous section we considered the direct effect of climate change on facilitation in established alpine plant communities. However, it is assumed that the majority of alpine plants are highly sensitive to temperature changes and will exhibit rapid upward migration toward higher elevations (Cannone et al., 2007; Lenoir et al., 2008; Pauli et al., 2012). Such upward migration to previously abiotic areas requires plant adaptations for primary succession, i.e., efficient propagule dispersal and the ability to cope with local environmental filters (Matthews, 1992; Frénot et al., 1998; Walker and del Moral, 2003; Caccianiga et al., 2006). From a climatic viewpoint, it is unlikely that conditions found in these newly colonized areas would be significantly different from those experienced by alpine plants in their original habitat. In contrast, soil properties are likely to differ, from old, organic alpine soils to a complete absence of soil, which is "the defining characteristic of the first stage of primary succession" (Walker and del Moral, 2003). Indeed, soils in newly colonized areas are expected at best to be mostly mineral in terms of their composition and poorly developed, except in scattered "safe sites" (Körner, 2003), thus increasing the level of stress for plants through the absence of resources (nutrients, water), as well as through reduced temperature buffering effects. Therefore, in line with the SGH, stronger facilitation among plants is expected to occur in these newly colonized areas (Stöcklin and Bäumler, 1996; Niederfriniger Schlag and Erschbamer, 2000; Jones and Henry, 2003; Erschbamer et al., 2008). However, given the rapidity of warming over the last four decades in alpine regions (IPCC, 2013), primary succession is very recent and these new areas display very little vegetation cover, comprising mostly of wind-dispersed species (Matthews, 1992) represented by juvenile individuals, which possess less potential to be nurse plants. For this reason, a number of observations made during the earliest stages of alpine primary succession have demonstrated the absence or low occurrence of nurse plants (e.g., Frénot et al., 1998). In the specific case of recently deglaciated areas, dispersal limitation and the slow growth rate of many alpine nurse plants (Ralph, 1978; Morris and Doak, 1998) may also limit the potential for facilitation among plants. To examine this hypothesis, recent data based on the Relative Interaction Index (RII; Armas et al., 2004) in the tropical high-Andes showed that (1) the cushion-forming species *A. aretioides* facilitated 50% of species in the surrounding alpine plant community at 4700 m a.s.l., and (2) the majority of these facilitated species were not present in a recently deglaciated area (0–13 yrs) directly above this site, where *A. aretioides* itself was absent (Cauvy-Fraunié, 2010; Anthelme et al., 2012; **Figure 3**). In comparison, seven out of eight species not facilitated by *A. aretioides* at 4700 m were present in the recently deglaciated site. These results suggest that, under the effects of accelerated warming, the absence of an important nurse species in a new alpine area targeted for upward migration will have a negative effect on plant diversity by impeding the establishment of associated plants (e.g., *Myrosmodes* sp., *Lupinus microphyllus*; **Figure 3**). This upholds the hypothesis that the future of (alpine) biodiversity could be partly dependent on facilitative and mutualistic interactions among organisms, required to avoid "chains of extinction" (Choler et al., 2001; Brooker et al., 2008; Bellard et al., 2012).

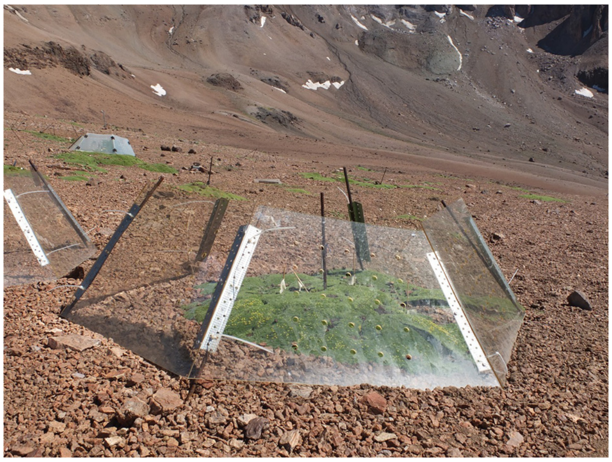

FIGURE 2 | Experimental site with open top chambers (OTCs) in central Chile. OTCs were implemented at sites with and without the cushion *Azorella madreporica* with the aim of examining the warming effects on facilitation by *A. madreporica* (Cavieres and Sierra-Almeida, 2012).

FUTURE RESEARCH ON MIGRATING COMMUNITIES

The few data available on facilitation among upward-migrating plants in alpine communities are in line with our second hypothesis of increasing facilitation in these new alpine areas. Nevertheless, knowledge gaps on plant–plant interactions in future alpine communities establishing at higher elevations are even more obvious than those affecting established alpine plant communities, explaining why we were unable to provide a quantitative review for this section. One of the reasons for this fact may be that experimental designs are more complex to set up in these areas, perhaps requiring soil removal instead of being based solely on the installation of OTCs. However, an interesting alternative could be to manipulate conditions through the addition of soil from lower alpine sites into the new alpine sites at higher elevations to examine the performance of species transplanted from the same lower site in a factorial experiment. Implementing this type of experimentation is a stimulating challenge for future

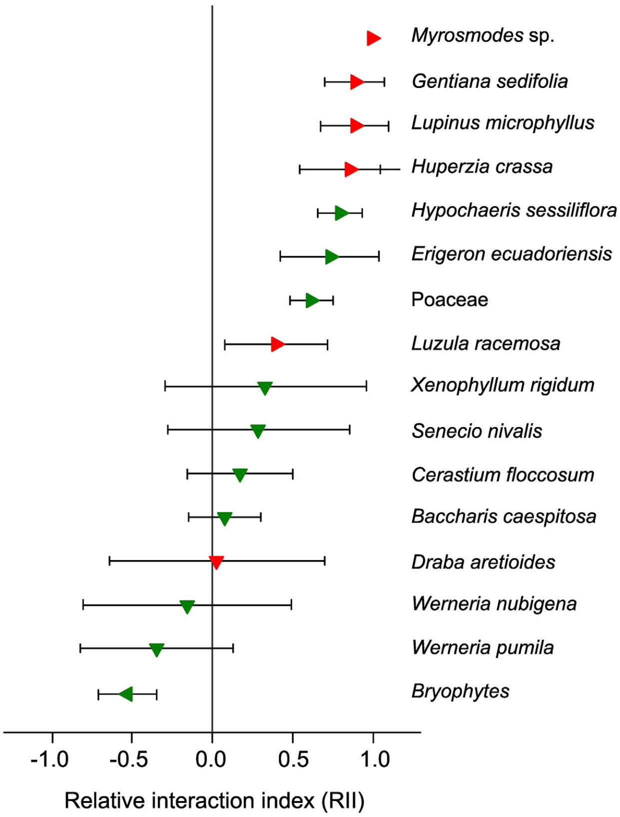

FIGURE 3 | Tropical alpine species observed in the superpáramo of Volcano Antisana and the outcome of their interactions with the cushion plant *Azorella aretioides* (Ecuador, 4700 m a.s.l.). RII > 0: species facilitated by *A. aretioides* (right-facing triangles); RII < 0: species inhibited by *A. aretioides* (left-facing triangles); RII not significantly different from 0: neutral interactions (downward-facing triangles; Anthelme et al., 2012). Red triangles indicate that the species were not observed in the adjacent, recently deglaciated site (between 0 and 13 years after glacial recession; Cauvy-Fraunié, 2010). Green triangles indicate that the species were observed in the recently deglaciated site. Error bars represent the 95% CI.

research. Knowledge gaps might also be indebted to the prevalence of a conservationist viewpoint of alpine plant communities, leading us to focus our research on established alpine communities threatened by changes rather than future communities establishing themselves at higher elevations. An interesting option to bridge this gap is to collect data in longer term experiments using permanent plots. This is the approach developed by the GLORIA network in alpine environments on the global scale (http://www.gloria.ac.at/) and it would be particularly relevant to take advantage of these designs to focus on the future of plant–plant interactions. Alternatively, using proglacial chronosequences as a space-for-time substitution approach to the study of climate change effects (Blois et al., 2013) may yield important and precise information on the role played by plant–plant interactions during accelerated upward migration of plants, wherever precise data on glacial retreat are available. Indeed, glacial retreat has been a continuous feature worldwide over the last 30–40 years and is documented precisely and regularly at an increasing number of glacial sites on various continents (Rabatel et al., 2013 and references therein). These data have been widely used to document processes of primary succession (e.g., Matthews, 1992; Erschbamer et al., 2001; Caccianiga et al., 2006). However, they have not explicitly considered changes in the direction and intensity of plant–plant interactions over recent decades.

ALPINE PLANT COMMUNITIES UNDER CLIMATE CHANGE: THE INFLUENCE OF FACILITATION

As a global interpretation of Sections Facilitation in Established Alpine Communities: a Bibliographic Review and Facilitation and the Upward Migration of Alpine Species, we propose an exploratory framework to predict the constraints applied to alpine plant communities under global warming, considering both established and migrating plant assemblages, and taking into account the role played by plant–plant interactions (**Figure 4**). It deals with two different paces of warming. Moderate warming refers to that experienced by Earth between the Little Ice Age (approx. 1650–1750 AD) and the recent acceleration of warming in the 1970s. Rapid, current warming refers to the period from the 1970s to the present day.

In established communities, alpine plants are expected to be impacted negatively by the effects of rapid warming, both directly through thermophilization (Gottfried et al., 2012), but mainly indirectly through net facilitation release (1) with existing neighboring plants (see review in **Table 1**) and (2) with species migrating from lower elevations (Gilman et al., 2010; La Sorte and Jetz, 2010; Chen et al., 2011). Nevertheless, earlier snowmelt may promote facilitative interactions among plants, at least in the short term (Wipf et al., 2006). At the same time, we do not expect moderate warming to have a significant impact on alpine species pools.

The diversity of upward-migrating alpine communities is expected to be negatively affected by both moderate and rapid warming. Under moderate warming this occurs through dispersal limitation (Garbarino et al., 2010), increasing soil harshness in comparison with the initial alpine site (Matthews, 1992; Stöcklin and Bäumler, 1996; Jones and Henry, 2003), and a deficit in the abundance/maturity of nurse plants (i.e., a deficit in facilitation among plants: Stöcklin and Bäumler, 1996; Niederfriniger Schlag and Erschbamer, 2000; Jones and Henry, 2003; Erschbamer et al., 2008). Moreover, existing data—mostly extracted from the first steps of primary succession after glacial recession—suggest that all these constraints should be exacerbated under rapid warming, i.e., stronger dispersal limitation (Matthews, 1992; Stöcklin and Bäumler, 1996; Erschbamer et al., 2001, 2008), aggravated soil harshness combined with a water availability deficit (Caccianiga et al., 2006; Sattin et al., 2009; Anthelme and Dangles, 2012), and a greater deficiency in the prevalence of nurse plants as a result of a shorter available time for them to recruit and establish in new areas (Frénot et al., 1998).

This overall pattern of alpine species impoverishment is largely dependent on (the absence of) facilitation among plants, thus evidencing the important role that plant–plant interactions may play in the future of alpine communities under the effects of climate change.

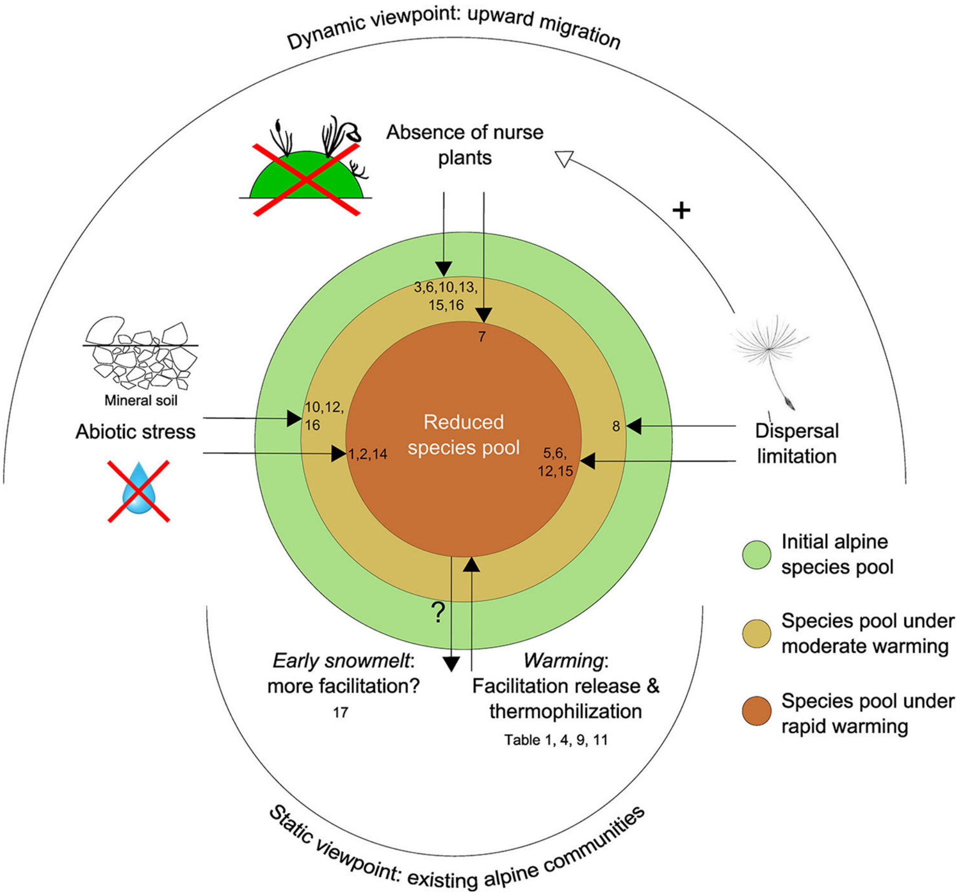

FIGURE 4 | Facilitation as a driver of alpine community structure under two different paces of warming, in established vs. migrating alpine communities. References: (1) Anthelme and Dangles, 2012; (2) Caccianiga et al., 2006; (3) Chapin et al., 1994; (4) Chen et al., 2011; (5) Erschbamer et al., 2001; (6) Erschbamer et al., 2008; (7) Frénot et al., 1998; (8) Garbarino et al., 2010; (9) Gilman et al., 2010; (10) Jones and Henry, 2003; (11) La Sorte and Jetz, 2010; (12) Matthews, 1992; (13) Niederfriniger Schlag and Erschbamer, 2000; (14) Sattin et al., 2009; (15) Stöcklin and Bäumler, 1996; (16) Walker and del Moral, 2003 (17) Wipf et al., 2006.

FACILITATION AND CLIMATE CHANGE ALONG LATITUDINAL GRADIENTS

DISTINCTIVE DRIVERS OF INTERACTIONS AT LOW vs. HIGH LATITUDES

There are two reasons for taking a closer look at plant–plant interactions along latitudinal gradients when addressing the future of alpine plant communities in the face of climate change: (1) patterns and mechanisms of interactions are expected to vary with latitude; and (2) the intensity of warming may also vary with latitude. A recent review of tropical alpine environments proposed that plant–plant interactions are under the control of a number of drivers, which are distinct from those found in extratropical alpine regions, including aseasonality, the absence of persisting snow cover, different plant life-forms, and the possible inversion of precipitation gradients. They are expected to generate both temperature and water stresses for plants at high elevation sites (Anthelme and Dangles, 2012). As mentioned earlier in the paper, snow cover duration and dry alpine environments are both expected to provide a different outcome with respect to interactions under the influence of climate change (Wipf et al., 2006; Michalet et al., 2014).

From a climatic viewpoint, despite the general assumption that future global warming will have a more severe impact in arctic regions (Nogués-Bravo et al., 2007; Loarie et al., 2009; IPCC, 2013), data extracted from wide latitudinal gradients suggest that warming may peak at higher elevations, closer to the troposphere, and at lower latitudes (Bradley et al., 2006; Thompson et al., 2011; **Figure 5**). This assumption is supported by the greater velocity of glacial retreat observed during the last few decades within the alpine tropics compared to the global scale (Rabatel et al., 2013). When superimposed on the latitudinal distribution of alpine ecosystems, these projections suggest that alpine ecosystems facing the strongest warming in the next few decades will be those distributed at lower latitudes, in the tropics and in the subtropics (**Figure 5**).

STATE-OF-THE-ART AND FUTURE RESEARCH ON THE EFFECTS OF LATITUDE ON FACILITATION

Existing data on plant–plant interactions along large alpine latitudinal gradients are also scarce, even without considering the effects of climate change. The first global-scale study did not

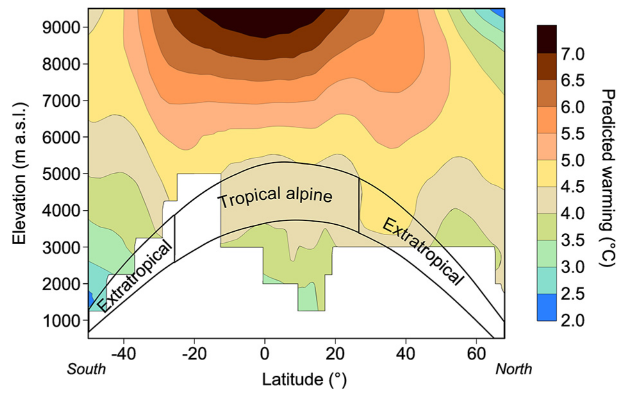

FIGURE 5 | Projected temperature warming along a latitudinal gradient and an elevation gradient, with a focus on alpine regions, worldwide [from 1990–1999 to 2090–2099, based on the 4th assessment report of the Intergovernmental Panel on Climate Change (IPCC)]. Modified from Körner (2003) and Bradley et al. (2006).

really consider low-latitude sites (mimimum: 26.5°S; Argentina) and did not provide any clear latitudinal pattern (Callaway et al., 2002). Taking advantage of the large latitudinal range of alpine environments in Chile, Cavieres and Badano (2009) used alpine cushions as neighboring species. They evidenced a facilitation peak at 41°S, which decreased—but not regularly—at lower latitudes. More recently, a global study across 78 alpine/arctic sites at all latitudes using cushion plants as neighboring species seemed to confirm this trend, with maximum facilitation observed at moderate- and high-latitude sites, whereas at low-latitude facilitation was found to diminish (Cavieres et al., 2014).

However, the review provided in Section Facilitation in Established Alpine Communities: a Bibliographic Review revealed that there are barely any data on changes in plant interactions with latitude under the effects of climate change. Indeed, most studies have been carried out in temperate and arctic regions (e.g., the Alps, Alaska, Scandinavia), whereas low-latitude alpine regions are largely overlooked. A recent review of nurse plant mechanisms on the global scale lends support to this view by evidencing a strong research gap on plant interactions in tropical (alpine) regions (Filazzola and Lortie, 2014). Only one study has been conducted in tropical alpine environments, supporting the general assumption that more competition will drive established communities, whereas more facilitation will drive the dynamics of upward-migrating communities (Almeida et al., 2013; **Figure 4**). Accordingly, although an overall pattern of decreasing facilitation with decreasing latitude seems apparent, there are insufficient empirical data to corroborate our third hypothesis that distinct variation in facilitation will be apparent at low and high latitudes under the effects of climate change.

Given the specific environmental charactersitics of low-latitude environments in comparison with high-latitude environments, an important challenge is to provide larger datasets for tropical alpine regions that include climate change designs along the lines of those described in Section Facilitation in Established Alpine Communities: a Bibliographic Review, at several points along large latitudinal gradients [e.g., similar to the gradients studied by glaciologists and climatologists from Alaska to Patagonia (Bradley et al., 2006)]. We expect such an approach to contribute positively to future projections of alpine biodiversity that take into account plant–plant interactions.

LONG-TERM FACILITATIVE EFFECTS BY NURSE PLANTS

PERSISTENCE OF BUFFERING EFFECTS AND ALPINE LIFE FORMS

Evaluating the real impact of facilitative processes on plant communities when considering restoration or conservation concerns remains a challenge because of the transient nature of interactions among plants, not only along stress or disturbance gradients, but also along temporal gradients (Bellard et al., 2012; Prévosto et al., 2012). In particular, both nurse plants and beneficiary species can alter the outcome of interactions because of ontogenic shifts (Callaway and Walker, 1997). Several studies have shown that the seedling stage is the best life stage for beneficiary species to be facilitated by nurse plants (see Callaway, 2007 and references therein). However, a recent study in an alpine region indicated that medium-sized individuals rather than seedlings or adults were more prone to facilitation (Le Roux et al., 2013), stressing the need for more explicit studies in this overlooked area (Armas et al., 2013).

In light of the rapidity of climate change affecting alpine regions, the transient nature of interactions is even more puzzling because interacting plants are expected to increase their growth rate (e.g., Hudson et al., 2011), thus modifying substantially the terms of the competition–facilitation equation (e.g., Adler et al., 2012). Nevertheless, many alpine plants develop life forms related to stress-tolerance strategies, i.e., a small size, slow growth rate, and are little sensitive to variations in resource availability (Grime, 1977; Cerabolini et al., 2010). Therefore, we hypothesize that some alpine nurse plants may act as long-term efficient facilitators for other alpine plants under the effects of climate change because their morphology, and thus their buffering effects on the microenvironment, will not change much with time. Two of the most abundant life forms in alpine regions are cushion-forming plants and tussock grasses (Hedberg and Hedberg, 1979; Körner, 2003). Even though tussock grasses have been reported to be highly competitive for resources in dry environments (Gómez-Aparicio, 2009), a number of studies have shown that, within an alpine context, both life forms may act as efficient nurse plants (Callaway et al., 2002; Catorci et al., 2011; Cavieres et al., 2014). We take these two life forms as distinctive cases in the following two paragraphs to explore how the predicted variation in their buffering effects on the microenvironment in the face of climate change may drive their nurse-related impacts on other plants in alpine regions (**Figure 6**).

Cushion-forming plants (hereafter referred to as "cushions") comprise 1309 species, the majority of which are alpine species (Aubert et al., 2014). They are slow-growing species that generate biotic substrate, thus "[engineering] their own environment, allowing the system to be less susceptible to direct changes in climate" (Benavides et al., 2013). The relatively regular lateral growth of cushions has enabled reasonably accurate estimations of their age at several hundreds to thousands of years (Ralph, 1978; Morris and Doak, 1998; le Roux and McGeoch, 2004). For these two reasons, cushions provide regular water, buffered

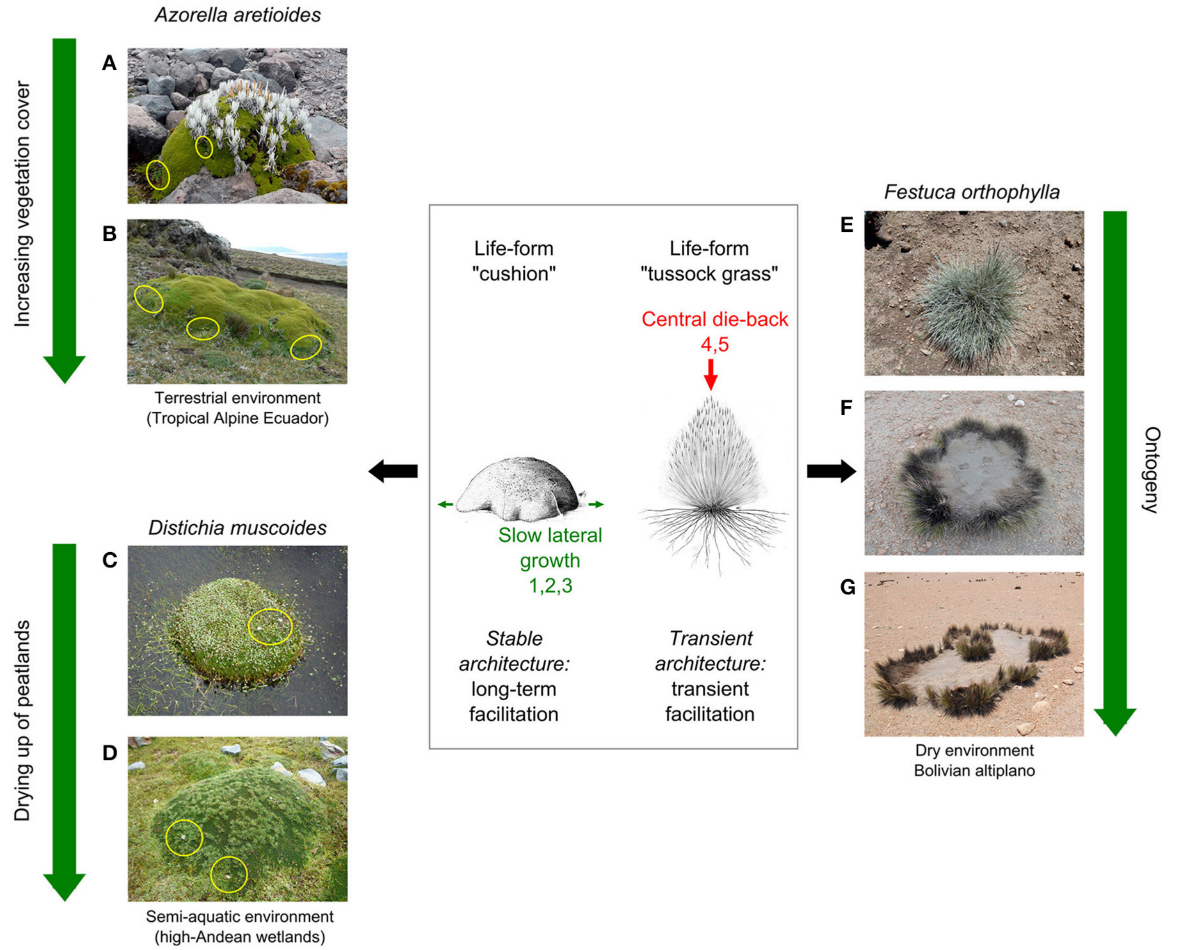

FIGURE 6 | Two distinct alpine life forms expected to generate two interaction outcomes under the effects of climate change. On the left, cushion-forming plants: **(A,B)** *Azorella aretioides* in terrestrial environments, with the alpine beneficiary *Hypochaeris sessiliflora* observed both during the first steps of primary succession and long after, when vegetation cover at the soil surface approaches 100% (red circles; Anthelme et al., 2012); **(C,D)** *Distichia muscoides* in semi-aquatic environments, with the beneficiary species *Gentiana sedifolia* (light blue flowers, yellow circles) and *Phylloscirpus deserticola* (light green cover on the cushion) observed even after the area dried up and was colonized by other plant assemblages (Loza Herrera et al., 2014). On the right, the tussock grass *Festuca orthophylla*: **(E)** high stem density in young individuals; **(F)** central die-back in mature individuals; **(G)** intraspecific colonization of the tussock center. (1) Ralph, 1978; (2) Morris and Doak, 1998; (3) le Roux and McGeoch, 2008; (4) Carteni et al., 2012; (5) Adachi et al., 1996. Drawings: Carlos Maldonado.

temperatures, nutrients and protection from wind for other plants (Cavieres et al., 2002, 2006; Nyakatya and McGeoch, 2008; Badano and Marquet, 2009; Anthelme et al., 2012), i.e., a buffered microenvironment that is expected to be profitable for other alpine plants in the long-term. In the Ecuadorian high Andes, a study on the positive effects of the cushion *Azorella aretioides* on the alpine community showed that the majority of species were facilitated at higher elevation (**Figure 6A**), whereas neutral or negative interactions prevailed at lower elevation, equivalent to a subalpine, tropical environment (Anthelme et al., 2012). However, even at lower elevation with high vegetation cover at the soil surface, a number of true alpine species such as *Huperzia crassifolia*, *Hypochaeris sessiliflora*, *Myrosmodes* sp. or *Oreomyrrhis andicola*—all facilitated at higher elevation—remained present and facilitated by *A. aretioides* (**Figure 6B**). This suggests that these species take advantage of the longevity and stability of *A. aretioides* to persist at this elevation, which is otherwise colonized by more competitive species from lower elevation, having migrated there because of warming (*Calamagrostis intermedia*, *Festuca* spp., *Chuquiraga jussieui*). Similarly, in the high-Andean wetlands of Bolivia, the dominant species *Distichia muscoides* provides a terrestrial, but water-saturated substrate for a very specific plant community, which is believed to be threatened by reduced water availability as a result of accelerated glacial recession, a direct consequence of warming (**Figure 6C**). However, the temporal stability of the cushion's structure and its engineering effect on the environment seems to make it possible for it to persist in the long-term—even if the wetland dries up—thus protecting an entire assemblage of associated plants, such as *Phylloscirpus deserticola* (**Figure 6D**; Loza Herrera et al., 2014). These two sets of empirical data, although partly speculative, are in line with the hypothesis that the long-term buffering effects of alpine cushions on the microenvironment provide a similarly long-term refuge for true alpine communities that are otherwise unable to cope with increased competition from species migrating from lower elevations.

Along with alpine cushions, tussocks are also long-living life forms (at least several decades; Catorci et al., 2011), but their morphology is much more variable throughout ontogeny. Unlike cushions, tussock stems develop vertically, reducing light and access to the substrate beneath for beneficiary species. Most of all, they frequently experience central die-back in mature individuals, likely because of intra-individual competition for water at their center (Cartenì et al., 2012; Couteron et al., 2014; **Figure 6**). This ontogenic pattern is cyclic, and new individuals or new ramets colonizing the center of tussocks can themselves experience central die-back, generating complex but structured distribution patterns (**Figure 6G**). Therefore, unlike young individuals, whose high density of stems make them highly competitive for other plants, mature tussock grasses such as *Festuca orthophylla* (**Figures 6E–G**) may provide microenvironments with higher nutrient content and reduced negative interactions, thus generating net facilitative effects (Catorci et al., 2011). As a consequence, facilitative effects provided by tussocks are transient, which seemingly does not make this life form a stable biotic refuge for other alpine species trying to escape the effects of climate change.

In summary, these data seem to corroborate our fourth hypothesis that some alpine plants may provide biotic refuges for other alpine species through their long-term, non-transient buffering effects on abiotic parameters in the face of climate change. Cushion plants, whose growth is particularly slow and regular, are examples of such species. Tussock grasses, whose growth is cyclic and irregular, are not.

LONG-TERM BUFFERING EFFECTS AND LOCAL MIGRATION OF ALPINE PLANTS

Evidencing the possible long-term facilitative effects of some alpine plants on their neighbors revealed a third migration option for established alpine plants under the effects of climate change, which is directly connected with facilitation among plants. Interestingly, each of these migration options is partially sustained by facilitation among plants (**Figure 7**). Upward migration is obviously the most widespread pattern observed and requires nurse plants to be successful (e.g., Lenoir et al., 2008; **Figures 4**, **7**). Nevertheless, up to 25% of species may experience significant downward migration, taking advantage of increased disturbance and corresponding increased transient facilitation with other plants in these areas (in line with the SGH; Lenoir et al., 2010). Interestingly, Scherrer and Körner (2010, 2011) identified a third option for alpine plant migration by demonstrating with thermal cameras that local variations in temperature related to microtopography in established alpine communities may exceed IPCC warming projections for the next 100 years. Thus, along with upward and downward migration, local migration may be an important fallback option for alpine plants. The long-term stable architecture of alpine cushions suggests that this local migration may take place not only because of microtopographical variation, but also because of the persistence of such life forms in established alpine communities under global warming, which will permit the presence of other alpine species via facilitative interactions (**Figure 7**). This assumption agrees with recent data evidencing the pervasive positive effects of alpine cushions on plant communities on a global scale (Cavieres et al., 2014).

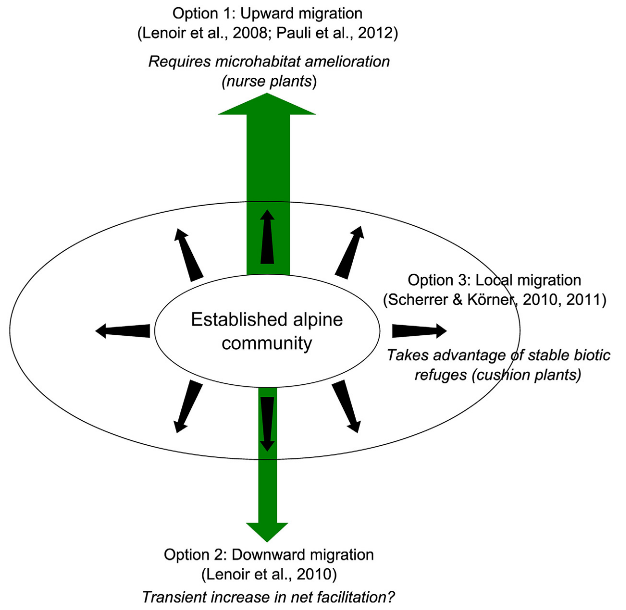

FIGURE 7 | Three migration scenarios for alpine plant communities under the effects of warming (upward, downward, local) involving three potential facilitative interactions among plants.

FUTURE RESEARCH ON THE LONG-TERM FACILITATIVE EFFECTS OF ALPINE PLANTS

Temperature buffering has been cited as an important facilitating mechanism by alpine cushion plants. Consequently, the effects of alpine nurse plants on temperature under climate change are predicted to be a crucial driver of the distribution of alpine species. Recent methodological advances in alpine regions have permitted the spatial representation of plant and soil surface temperatures using thermal cameras. Results have demonstrated that variations in surface temperature and root temperature under global warming are not necessarily correlated with atmospheric temperature (Scherrer and Körner, 2010). Accordingly, measuring the surface temperature of potential nurse plants with thermal cameras rather than the air temperature should provide a more accurate explanation for the patterns of observed plant–plant interactions. Repetition of these spatially explicit measurements at different elevations, as a space-for-time substitution of global warming (Blois et al., 2013), may reveal interesting temperature patterns, which—if correlated with structured patterns of plant interactions—could provide an interesting insight into the future direction and intensity of plant–plant interactions

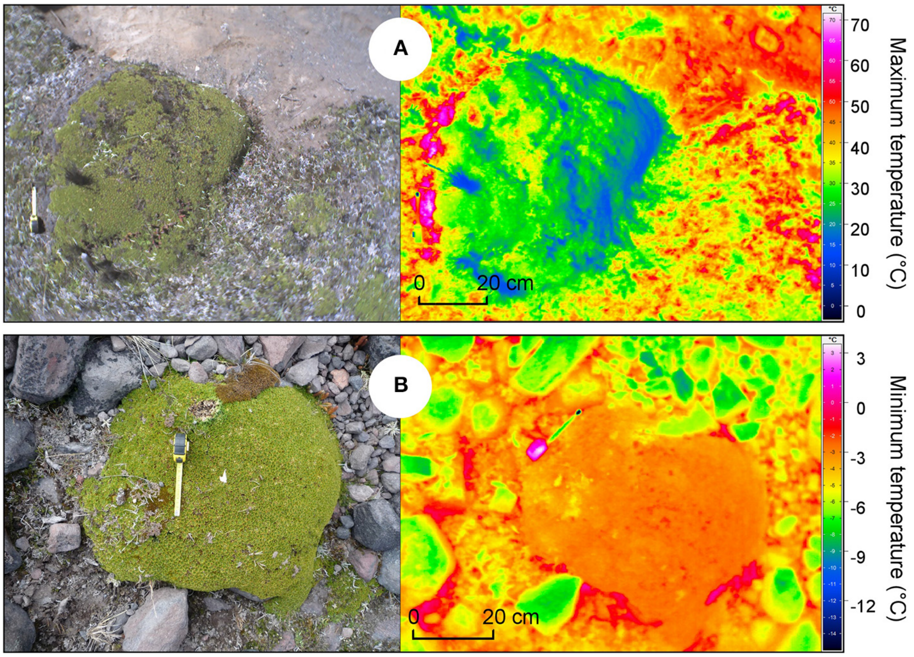

FIGURE 8 | Spatial representation of the buffering effects of the cushion-forming plant *Azorella aretioides* on temperature (Ecuador, 4700 m a.s.l.). (A) *A. aretioides* mitigates maximum temperature during the day; **(B)** *A. aretioides* increases minimum temperature at night (unpublished data; Thermographic System VarioCAM® hr—head 680/30 mm GigE).

in alpine regions. Given the relatively smooth, well-delineated surface of alpine cushion plants, these life forms are expected to be important phytometers for such a purpose. Preliminary results in the High-Andes with the cushion plant *A. aretioides* provide strong agreement with the hypothesis that cushions reduce the maximum temperature during the day, especially in comparison with rocks, bare soil, and dead cushion parts (**Figure 8A**), and increase the minimum temperature at night, (**Figure 8B**).

Combining these data with the spatial representation of other abiotic data such as humidity, nutrient availability and topography may provide powerful interpretations of the mechanisms underlying plant–plant interactions at the landscape scale in the face of rapid climate change in alpine regions.

AUTHOR CONTRIBUTIONS

Fabien Anthelme, Lohengrin A. Cavieres, and Olivier Dangles contributed to the conception, the structure and the writing of the manuscript.

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REVIEW
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Species coexistence in a changing world

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The consequences of global change for the maintenance of species diversity will depend on the sum of each species responses to the environment and on the interactions among them. A wide ecological literature supports that these species-specific responses can arise from factors related to life strategies, evolutionary history and intraspecific variation, and also from environmental variation in space and time. In the light of recent advances from coexistence theory combined with mechanistic explanations of diversity maintenance, we discuss how global change drivers can influence species coexistence. We revise the importance of both competition and facilitation for understanding coexistence in different ecosystems, address the influence of phylogenetic relatedness, functional traits, phenotypic plasticity and intraspecific variability, and discuss lessons learnt from invasion ecology. While most previous studies have focused their efforts on disentangling the mechanisms that maintain the biological diversity in species-rich ecosystems such as tropical forests, grasslands and coral reefs, we argue that much can be learnt from pauci-specific communities where functional variability within each species, together with demographic and stochastic processes becomes key to understand species interactions and eventually community responses to global change.

Keywords: competition, facilitation, global change, functional traits, heterogeneity, intraspecific variability, climate change

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INTRODUCTION

Species composition of a local community is the result of several processes and factors that act at different scales, none of them being mutually exclusive. This encompasses from features and processes that act at global and regional scales, such as randomness, historical patterns of speciation, extinction, migration as well as dispersal processes, to abiotic factors (physical constraints of the environment) and biotic interactions (both positive and negative) that act at local scale. These factors, known as hierarchical filters, act from broad to fine spatial scales to impose rules on community assembly (Götzenberger et al., 2012). There are numerous theories about these filters and the coexistence mechanisms involved in the composition of species in a community. In this article we focus on those acting at local scales (**Figure 1**), but we also refer to broader scales and the corresponding interactions since they are key to understand regional and global species diversity.

Biological diversity is about species interactions inter alia, and it is commonly limited by competitive exclusion and sometimes fueled by positive relationships. Competitive exclusion has

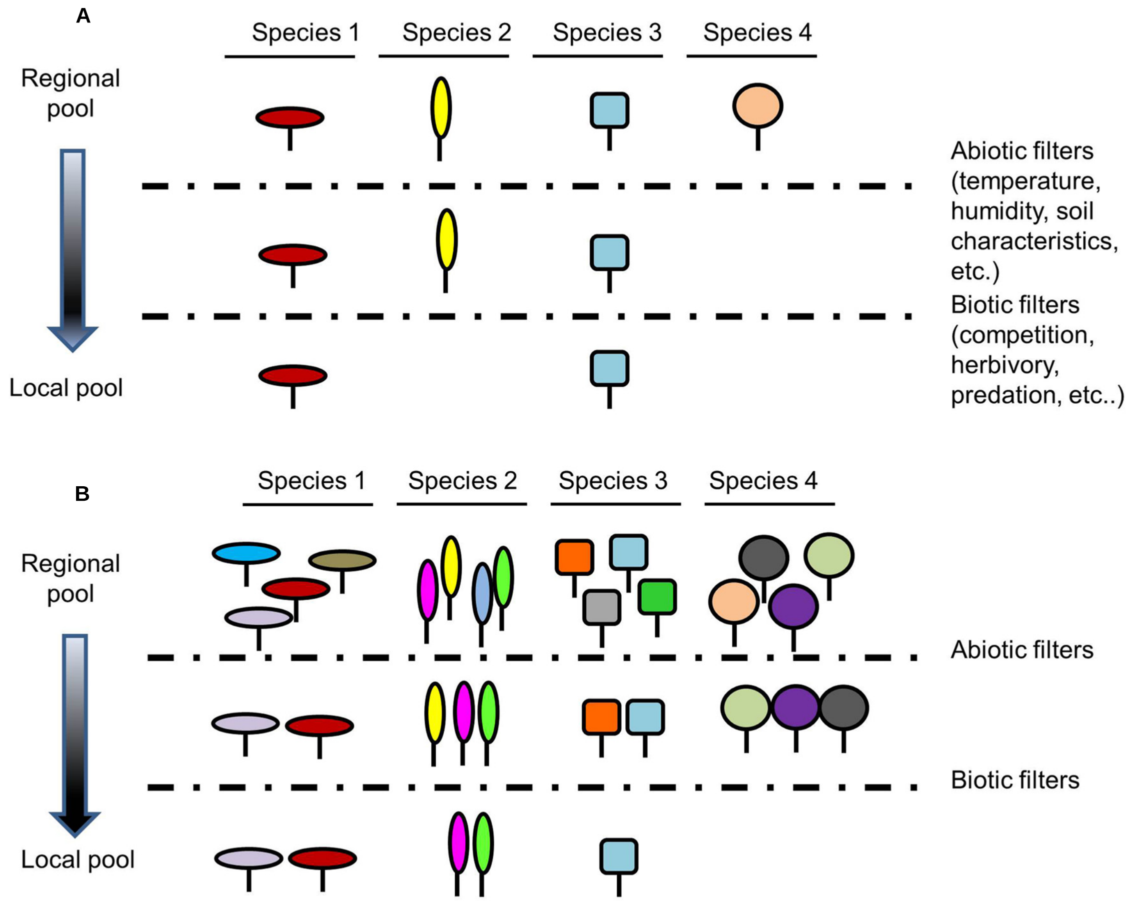

FIGURE 1 | Influence of intraspecific variability in the filtering of potential species integrating a community. (A) classical community assembly theory without taking into account intraspecific variability and **(B)** community assembly theory incorporating intraspecific variability. Species with mean trait values matching the abiotic requirements and being either ecologically different or capable of tolerating competition will contribute to the eventual community. By incorporating intraspecific variability, more species will pass biotic and abiotic filters because they are able to adjust by phenotypic plasticity or simply because they are genetically variable so more species could join the community in **(B)** than in **(A)**. Each shape represents a species and each color represents a given trait value within a species. Dashed lines represent abiotic and biotic filters.

a crucial role in structuring communities and has therefore prompted intensive ecological research over decades (Pianka and Horn, 2005). Competition has both an evolutionary and an ecological role since it increases diversity through speciation (Brännström et al., 2012) and regulates species diversity through species interactions (Chesson, 2000). Classical coexistence theories establish that each species inhabits a particular niche, involving a given combination of abiotic and biotic factors, where it outcompetes the rest of the species in the local pool (i.e., niche theory; Grinnell, 1917; Gause, 1934). Under this premise, niche overlap penalizes worse competitors, which results in their exclusion from a community, and supports that species coexist by being functionally different and by exploiting different niches (Hutchinson, 1959). If true, the total number of species in an ecosystem is thought to be proportional to the total range of the environment divided by the niche breadth of the species (MacArthur and Levins, 1967). In contrast, neutral theory (Hubbell, 2001) assumes that individuals and species are ecologically interchangeable and therefore equivalent in their competitive ability, i.e., none of the species shows an advantage or disadvantage over the others. According to the neutral theory, random processes, stochastic events, and equivalence between opposite forces are the drivers of population dynamics and species coexistence (Bell, 2000; Hubbell, 2001, 2005; Götzenberger et al., 2012). However, these theoretical frameworks seem insufficient to explain species coexistence in many natural ecosystems and numerous discrepancies have been found between theoretical predictions from classic niche theory and empirical studies (Nathan et al., 2013).

Here we review the theory about the mechanisms underlying the maintenance of species coexistence. Although conclusions and main concepts apply to all sort of living organisms, we have placed special focus on plant communities and, hence, on plant species coexistence and diversity. We give special attention to concepts like competition, facilitation, ecological differences among species, intraspecific variability and environmental heterogeneity. In each section, we discuss how global change may affect species coexistence through modifications in important biotic and abiotic factors. The consideration of all global change factors potentially affecting coexistence would largely exceed the limits of this article so we have focused on the best studied ones and on those illustrating different responses and cascade effects on community dynamics and species interactions. We include an analysis of biological invasions, as a large and unique ecological and evolutionary experiment of coexistence. Also, we encompass the particular case of species coexistence in pauci-specific systems, which complement the better studied cases of tropical, hyperdiverse systems.

COMPETITION AND RELATED MECHANISMS TO EXPLAIN SPECIES COEXISTENCE

A number of alternatives have been proposed to explain coexistence and diversity when classic niche theory fails (Barot, 2004; Wildová et al., 2012). Under this emergent scenario, classic ideas on competition are being reshaped in a more mechanistic framework giving new perspectives that reconcile neutral and niche theories (Adler et al., 2007), often treated as mutually exclusive explanations.

This new mechanistic framework is explicitly addressed by combining the two concepts of Chesson's (2000) framework: the so-called "niche differences" and "fitness differences." Note that fitness is used as an ecological term, referring to the average competitive ability of a species, and not in an evolutionary context. Although complementing niche theory, niche differences do not determine the outcome of interactions alone. They are only a stabilizing mechanism favoring coexistence by limiting species abundance when they rise to dominance and buffering them against exclusion when they become rare (Adler et al., 2007). Differences in fitness favor dominance, and, in the absence of niche differences, they determine the species that exclude the rest. The key message of Chesson's (2000) framework is that the outcome of species interaction is jointly determined by the relative strength of niche differences versus fitness differences between species. In this context, coexistence will be fostered when niche differences overcome fitness differences (**Figure 2**).

This conceptual framework is now raising new questions among ecologists. For instance, since plants have a finite number of potentially limiting resources, the chances to overlap in their niches are in principle rather high (Wildová et al., 2012), particularly when compared to other biological groups such as animals. Is species coexistence therefore maintained in plants because small niche differences overcome small fitness differences or are high levels of niche differentiation still needed? Moreover, many species are dominant or even exclude the rest of the species at a given location, whereas they are inferior competitors at other locations. This opens the question as to what extent are spatially and temporally heterogeneous landscapes together with a large intraspecific variation in functional traits more important for the maintenance of species diversity than average species features and interactions. Giving responses to this kind of questions can undoubtedly advance our basic understanding of species coexistence. But equally important, they can also serve to predict how biological diversity will face a globally changing world.

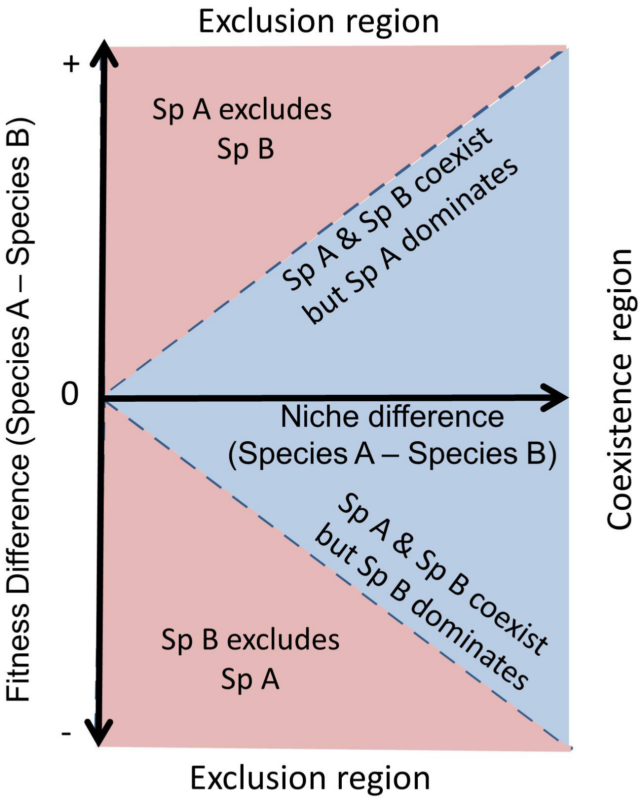

FIGURE 2 | A theoretical scheme of coexistence and competitive exclusion between two species. If niche differences between competitors are greater than their fitness asymmetries then both species will show stable coexistence (blue region). In contrast, if fitness differences are greater than niche differences, then the species with higher fitness will exclude the other (red region). Fitness differences also determine which species dominates under stable coexistence. Figure adapted from MacDougall et al. (2009).

Whether or not global change drivers are promoting differences among species in niche availability, in competitive ability or in a combination of both is crucial for understanding the evolution of plant communities in terms of diversity and coexistence as well as in terms of ecosystem functioning (**Table 1**). As a straightforward rule and because niche differentiation tends to stabilize coexistence, species diversity and niche diversity would tend to be correlated as classic niche theory proposes. However, when considering fitness differences possible complex changes may occur. For instance, the reduced competitive ability of the dominant plant species due to lower precipitations during spring (Clark et al., 2011), or due to the interactive effect of rainfall variability with soil pathogens (Gómez-Aparicio et al., 2012) can be dramatic for the affected species up to the point of their extinction at local scales. However, by eliminating the dominant species, rare species could persist, resulting in a community with increased diversity as Mariotte et al. (2013) showed in a drought experiment in grasslands of central Europe. In the same way, increases in fitness instead of reductions

TABLE 1 | Effects of global changes drivers on the outcome of species interaction through their effect on niche and fitness differences.

Global change driver	Effect on niche differences	Effect on fitness differences	Examples
Climate change	Increased climate variability can increase niche differentiation by promoting species with contrasted phenotypes.	New climate regimes possibly change the species hierarchy according their competitive ability. Dominant species become less competitive and subordinate species increase their dominance.	Sherry et al. (2007), Willis et al. (2008), Angert et al. (2009), Mariotte et al. (2013)
Nutrient pollution	Increase in nutrients (N, P) is reducing environmental heterogeneity and thus the chances of species to exploit resources from different niches.	A few species are benefiting from these more homogeneous environments leading to a few species outcompeting the rest. Other species are excluded because they cannot tolerate the new environmental conditions.	Reich et al. (2001), Stevens et al. (2004), Wookey et al. (2009)
Land use change	Novel ecosystems and intense landscape transformations is homogenizing the environment and reducing niche differences within communities. Among communities, land conversion is producing contrasting novel habitats increasing niche differentiation among species at large geographical scales.	Similar effects to nutrient application to agricultural systems. The competitive ability of a few species is dramatically increased, while other species are not able to survive. This reduces the diversity among and within communities.	Hobbs et al. (2006)
Biological invasions	Exotic species with contrasted phenotypes are able to exploit different resources increasing niche differentiation with respect to the resident community. Exotic species with similar phenotypes would reduce niche differentiation and increase niche overlap.	Although most of the introduced species fail to survive and invade because they cannot tolerate the new environmental conditions where they are introduced, successful invaders tend to possess traits that maximize competitive ability for a given quantity of resources.	Strauss et al. (2006), Funk et al. (2008), MacDougall et al. (2009), van Kleunen et al. (2010), Fridley (2012), Godoy and Levine (2014)

would also produce dominance of a single group of species, thereby reducing diversity, as it is the case for the interactive effect of climate change and biological invasions (Vitousek et al., 1997). However, diversity can be increased by equalizing fitness differences if the increase in fitness is for the inferior competitors (Gilman et al., 2010). Extinction of dominant species under extreme events or under intense pressure of global change drivers is very unusual since there are many mechanisms by which dominant species can persist with minimal community changes (Lloret et al., 2012). Although this has been less often reported, changes in species fitness can also reduce fitness differences among competitors reducing the likelihood of competitive exclusion. For example, at the edge between alpine and subalpine vegetation, climate warming is decreasing species fitness of the alpine species but increasing the fitness of the subalpine ones, resulting in an increased diversity at the ecotone (Parolo and Rossi, 2008). Atmospheric CO_2 enrichment can directly affect species interactions by increasing the fitness of species able to accelerate their growth rates in such enriched atmospheres, but there are still many knowledge gaps on such effects (Busch, 2015).

The discussion of the impact of global change on species persistence can also be extended to species abundances. Even minor changes in the mechanisms and processes determining coexistence can result in a great impact on species abundances as revealed by simulations based on microorganism traits and demography (Fox, 2012). Dominant or abundant species may exhibit large changes in their abundances despite small niche differences as a consequence of many stabilizing processes operating at different time and spatial scales (Lloret et al., 2012; Yenni et al., 2012). Thus, high competitive ability does not necessarily confer high abundance, particularly under changing or patchy environmental conditions, and even very small niche differences can dwindle the theoretical correlation between adaptive traits and abundance (Fox, 2012).

FACILITATION

Ecological research has mainly focused on competition when referring to species interactions and coexistence. Fitness differences, commonly related not only to the ability to produce offspring but also to the response to competition, reflect the net effect of competition and interspecific facilitation, with coexistence being prompted by an increase in fitness of rare, benefited species. Indeed, facilitation has been widely recognized in recent decades to be an important mechanism for maintaining community diversity and structure, particularly in plant communities (Callaway, 2007). Bruno et al. (2003) integrated facilitation into the niche theory highlighting its potential to increase the realized niche of the species. More recently, McIntire and Fajardo (2014) detailed in an extensive review the mechanisms by which facilitation may increase diversity and coexistence, including (1) stress amelioration, (2) novel habitat creation, (3) increased habitat complexity (i.e., heterogeneity) for a given area, (4) increased access to resources, and (5) service sharing such as pollination or dispersal efficiency.

Indeed, failure to incorporate these positive interactions likely limits our understanding of ecosystem functioning and responses to climate change (see Brooker et al., 2008, for a review). Positive interactions are thought to increase in importance when environmental conditions are harsher (see examples in **Figure 3**) becoming, thus, potentially more intense under current and future global changes (Michalet et al., 2006). This increase has been found in alpine and arctic habitats, where plant performance is limited by cold temperatures (Cavieres et al., 2014); in Mediterranean ecosystems subjected to intense and frequent drought events, and in other systems where survival or growth are

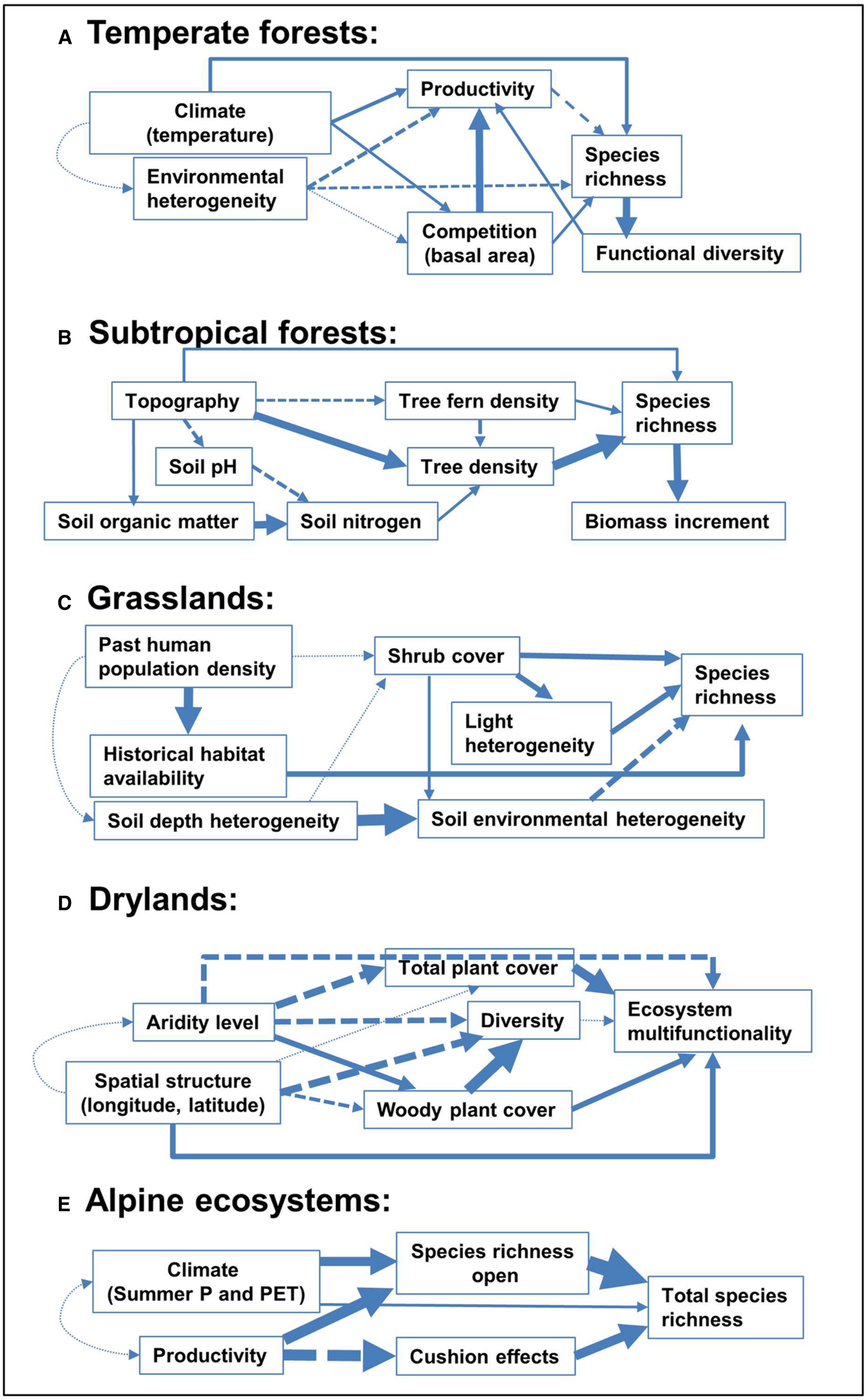

FIGURE 3 | Representation of direct and indirect pathways relating abiotic and biotic factors with diversity. We show examples of five study systems, corresponding to **(A)** temperate forests, modified from Paquette and Messier (2011), **(B)** tropical forests, modified from Yasuhiro et al. (2004), **(C)** grasslands, modified from Gazol et al. (2012), **(D)** drylands, modified from Soliveres et al. (2014), and **(E)** alpine ecosystems, modified from Cavieres et al. (2014). Single arrows represent causal paths, where thickness is proportional to the path coefficient (solid: positive, broken: negative, dotted: non-significant). Interlinked influences of landscape conditions and local environmental factors are explaining species richness in contrasted biomes such as subtropical forests and temperate grasslands. However, diversity and coexistence are usually dependent on distinct factors in each biome (i.e., competitive exclusion is more relevant in temperate forests, whereas facilitation mediated by woody cover or cushion effects are more important in drylands and alpine ecosystems, respectively).

limited by pervasive strong winds or excessive irradiance (Gómez-Aparicio et al., 2005; Cavieres and Badano, 2009; Fajardo and McIntire, 2010). Moreover, shifts from competition to facilitation at increasing stress have been demonstrated (e.g., Gross et al., 2013) despite exceptions and controversy (Maestre et al., 2009). Evidence also exists regarding the role of facilitation in milder environments (Holmgren and Scheffer, 2010; Granda et al., 2012), which brings about the broad prevalence of positive interactions and makes it clear that their effects on species coexistence, and thus on community diversity are likely wider than initially expected (Holmgren and Scheffer, 2010; McIntire and Fajardo, 2014).

The benefits of positive interactions on species composition have been addressed in a number of studies at local (Choler et al., 2001; Maestre et al., 2003; Cavieres and Badano, 2009) and, more rarely, regional or global scales (Valiente-Banuet et al., 2006; Cavieres et al., 2014). Cavieres et al. (2014) demonstrated in an extensive study using data sets across five continents that facilitation on cushion-dominated communities does not only enhance local but also global diversity, being as important as climatic drivers for the diversity of alpine ecosystems. If facilitation can have positive effects on species diversity, the opposite has also been demonstrated for aquatic organisms in stream mesocosms, where changes in species diversity altered the probability of positive species interactions, resulting in disproportionately large changes in the functioning of the study ecosystem (Cardinale et al., 2002). In plant communities, to our knowledge, this influence of species diversity on facilitation has rarely been addressed, although facilitation has been found to increase the phylogenetic diversity of the community (Valiente-Banuet and Verdu, 2007). Moreover, studies showing facilitation when strong niche overlap is present (Fajardo and McIntire, 2011), should shift our way to understand the species interactions. All these evidences demonstrate that facilitation is a ubiquitous driver of species diversity.

Global change impacts have been shown to be mitigated by facilitative interactions, including amelioration of climatic stress (Soliveres et al., 2011), reduced invasibility of communities by alien species (Bulleri et al., 2008) and increased survival, colonization or growth in habitats subjected to changes in land use (Gimeno et al., 2012). These processes allow for subordinate species, rare species or species with a low capacity to tolerate stress to survive thanks to the reduction of the environmental disturbances or intensity of the abiotic stress or predation (Hacker and Gaines, 1997). As a result, the biotic effects of nurse species should be combined with the nature and extent of environmental change to explain global patterns of species coexistence and to predict the effects of global change.

FUNCTIONAL TRAITS, PHYLOGENETIC RELATEDNESS, AND COMMUNITY ASSEMBLY

Ecological differences among species are based upon their functional traits, which are expected to provide niche and fitness differences (HilleRisLambers et al., 2012; Kraft et al., 2015). Some key functional trait differences between plant species that stabilize coexistence by niche partitioning include differences in rooting depth, phenology, responses to environmental gradients such as light or pH and the specificity of the interactions with host-specific pathogens (Grubb, 1977; Rathcke and Lacey, 1985; Liu et al., 2012). Traits related to fitness differences are often associated with the ability to deplete a shared limiting resource (Harper, 1977; Tilman, 1987), which can be for instance height and size in light-limited environments, or root density and the efficiency to acquire nitrogen and phosphorous in poor soils (Ojeda et al., 2010; Hill et al., 2011).

Trait-based predictions of future changes in biodiversity can be carried out by identifying the functional mechanisms that generate niche partitioning and fitness differences (Adler et al., 2013). Many environmental changes involve altered supply of limiting resources. In the case of nitrogen availability, for example, direct supply coming from N deposition is favoring non-N fixers over N-fixers, grasses over legumes, and C3 grasses over C4 grasses (Reich et al., 2001; Stevens et al., 2004; Wookey et al., 2009). Some responses to global change may be more difficult to predict because they involve change in both niche and fitness differences between species. A clear example is the change on plant phenology due to increasing temperatures. Hotter days during spring are advancing the timing of flowering and leafing (Peñuelas and Filella, 2001; Wolkovich et al., 2012), but at the same time, hotter days during summer for some ecosystems such as temperate prairies are splitting species toward an earlier and a later phenology community (Sherry et al., 2007). Because earlier activity is associated with a fitness increase (Verdú and Traveset, 2005), we can expect that species advancing their phenology faster will exhibit a fitness advantage, which could destabilize coexistence. However, separating the temporal niche into two contrasted phenologies will act as a stabilizing mechanism. Whether species coexistence is maximized or species with earlier phenologies are favored will depend on (i) which phenological change is dominant and (ii) how strong these phenological changes link to niche and fitness differences. Interestingly, climate change has modified the phylogenetic pattern of temperate fields, wetlands, and deciduous forests in the last 150 years (Willis et al., 2008) reducing the abundance and presence of those clades that could not adjust flowering phenology in response to temperature changes. Because flowering time correlates with species fitness (Godoy and Levine, 2014), it is likely that the patterns of exclusion are due to changes in fitness differences between clades.

The niche occupied by a species is defined by several functional traits in response to simultaneous stressors operating at different temporal and spatial scales, referred to as the multidimensional niche (Hutchinson, 1957). Despite its intrinsic complexity, this multifunctional information should be incorporated into models that forecast future species distribution in response to climate change (e.g., Kearney and Porter, 2009). The difficulties associated to the notion that several traits are involved in species coexistence have moved researchers to look for other approximations that can simplify this complexity. Because phylogenies reflect the evolutionary history of competing species and at least in part their ecological capabilities, it is expected that species phylogenetic relatedness informs on the main ecological process involved in the assembly of the community (Ackerly, 2003). The use of coexistence theory is refining the common expectation from classic niche theory that competitive exclusion leaves coexisting species more evenly spaced across the phylogeny than expected by chance from the regional species pool because closely related species tend to share a similar niche (Webb et al., 2002). Mayfield and Levine (2010) suggested that phylogenetic relatedness may also reflect differences in competition among species, with competitive exclusion leaving coexisting species more phylogenetically clustered than expected by chance. Mayfield and Levine (2010) concluded that competition could have a contrasting role for the phylogenetic structure of communities and that the outcome can be predictable with a mechanistic understanding of how phylogeny determines the niche and fitness differences between competitors. This theoretical explanation, albeit simplistic, can contribute to detangle mixed results (clustering and overdispersion) from previous work on phylogenetic competition experiments (Duncan and Williams, 2002; Maherali and Klironomos, 2007; Violle et al., 2011; Allan et al., 2013; Bennett et al., 2013; Narwani et al., 2013), and it can serve also to understand why random phylogenetic patterns as well as closely related species coexist together in many natural communities (Godoy et al., 2014). For instance, a puzzling finding in many tropical forests is the substantial contribution of a small number of species-rich plant genera to the total pool of species (the so called species swarms). In the case of the understory shrubs of the genus *Psychotria* in Panama, one of the scant ecological studies of these species swarms, congeners were found unlikely to exclude one another because resource availability was determined largely by asymmetric competition with the overstorey since within the understory *Psychotria* shrubs had similar competitive abilities (Sedio et al., 2012).

Functional traits are being increasingly considered for understanding climate change impacts by their inclusion in dynamic global vegetation models (DGVMs). DGVMs are powerful tools to test ecological theories and they are actually incorporating new concepts arsing from community ecology and coexistence theory (Scheiter et al., 2013). Despite increasing refinements there is a lack of a comprehensive analysis of the direct impacts of trait variation on global vegetation distribution and dynamics. Results by van Bodegom et al. (2013) have shown a great predictive ability of these models when they account for just a few relevant traits. We argue that even higher predictive ability could be achieved if intraspecific trait variability is included, as discussed in Valladares et al. (2014b).

INTRASPECIFIC TRAIT VARIABILITY

Species functions have been primarily defined on the basis of the mean values of their functional traits (**Figure 1A**), ignoring the extensive intraspecific variation typically found for most traits (**Figure 1B**). In fact, the contribution of intraspecific trait variability to trait-based coexistence theory has been underestimated over decades (Albert et al., 2010; Mitchell and Bakker, 2014). As a result of this research gap, an increasing number of studies have underlined the importance of incorporating information of intraspecific trait variation as a driver of species coexistence and community dynamics (Bolnick et al., 2011; Courbaud et al., 2012; **Figure 1B**). A study from forests in the southeastern of the United States revealed that the variation among the individuals within the study populations generated different distributions and responses to the environment among species, while the mean values for the corresponding populations did not differ (Clark, 2010). Lichstein et al. (2007) investigated the potential for intraspecific individual variation to maintain species coexistence through the use of a two species model assigning to each species a random independent competitive ability. These simulations showed that if the density of individuals competing for an open area is high, species with a large variance in competitive ability are favored, whereas the reverse is true if density is low. If there is an interspecific mean-variance competitive ability trade-off (e.g., one species competes against a second species that has a lower mean but a higher variance in individual competitive ability), stable coexistence can be expected over a range of intermediate densities. A superior vs. an inferior species (e.g., different means but the same variance in individual competitive ability) are expected in the absence of such a trade-off, and intraspecific variation would blur differences among species and the dynamics would follow the neutral case expectations. Even though Lichstein et al. (2007) showed that intraspecific variation can facilitate coexistence, they consider that it could play only a minor role for maintaining diversity in many real communities, which needs to be further explored.

Several studies have shown changes in the intraspecific variation of plant functional traits in response to new environmental conditions and new selection pressures resulting from global change drivers. For example, not only species turnover but also, and highly significant, intraspecific trait variability was found to be key in the functional response of alpine plant communities to drought (Jung et al., 2014).

An important source of intraspecific trait variability with key implications for population differentiation and local adaptation is phenotypic plasticity (Valladares et al., 2014b). Phenotypic plasticity can be defined as the ability of a genotype to show variable phenotypes in response to different environments (Garland and Kelly, 2006; Valladares et al., 2007). It has been widely recognized as a mechanism to cope with spatial and temporal heterogeneity, thereby avoiding migration or extinction of organisms under highly variable or increasingly distressed conditions (Matesanz et al., 2010; Nicotra et al., 2010). Jung et al. (2010) studying the role of the intraspecific trait variation on species assembly in grassland communities distributed along a flooding gradient found evidence that plasticity in resource use at the population level was an important mechanism of niche differentiation among plants. The promotion of species coexistence through resources partitioning have also been supported by Callaway et al. (2003), Miner et al. (2005), and Ashton et al. (2010). The lack of consistent patterns across lineages and geographical ranges together with the scarcity of sound empirical studies is challenging the inclusion of phenotypic plasticity in species distribution models used to forecast biodiversity under global change scenarios (Valladares et al., 2014b). As already noted by Pearman et al. (2010), species distribution models improve their results when incorporating within-species variation.

However, and despite the importance of intraspecific trait variability, its inclusion in trait-based coexistence theory remains a topic open to discussion (Kraft and Ackerly, 2009; Lake and Ostling, 2009; Bolnick et al., 2011). Albert et al. (2011) proposed a guideline on when intraspecific trait variability should be taken into account in ecological studies. The sequential steps of this guideline were: (1) whether the study explicitly encompasses intraspecific trait variability, such as evolutionary studies interested in trait or niche evolution; (2) the spatial scale of the study, with local studies typically more concerned with intraspecific trait variability; (3) the way the study species were chosen, i.e., species (e.g., few focal species) vs. site (e.g., all species within community) centered studies; in the former intraspecific trait variability is central, while in the latter the species turnover effect could be higher than the intraspecific trait variability effect. To decide upon the importance of including intraspecific trait variability in the case of site-centered studies, one more question still needs to be answered, (4) whether the study is interested in effect or response traits; in the latter case intraspecific trait variability is clearly central, while in the former case it might be omitted. Intraspecific trait variation seems appropriate to unify classic coexistence theory and evolutionary biology with recent trait-based approaches. For example, including this variation source in a hierarchical Bayesian model rendered accurate and realistic predictions and avoided some of the criticisms associated with some trait-based community assembly models (Laughlin et al., 2012).

ENVIRONMENTAL HETEROGENEITY AND DYNAMIC MOSAICS

Spatial heterogeneity can have a strong impact on species coexistence (**Figure 3**). In heterogeneous environments, species can be segregated in space according to their niche preferences (e.g., resource requirements). Classical examples include for instance differences in which chemical forms of nitrogen compounds are uptaken by tundra species (McKane et al., 2002). This prediction, inspired by the classical niche theory and contrary to the neutral theory, has been proven to promote coexistence in tropical forests over a wide sample of biogeographic conditions (Brown et al., 2013). Model simulations reveal the potentially important role of heterogeneity and its complex and delicate interplay with dispersal in mediating long-term outcomes of species coexistence (Schreiber and Killingback, 2013). For instance, when resource-rich patches are formed by an engineering species, habitats for species with high dispersal capacities are provided, allowing a successful colonization by these other species and their eventual coexistence with the engineering species. This spatial self-organization phenomenon has been reported by Nathan et al. (2013) using a mathematical formulation. While many studies recognize that spatial heterogeneity promotes species diversity, high species diversity itself can also increase spatial heterogeneity for factors like light (each species canopy intercept light differently) or soil water and nutrients (each species explores below ground resources differently), which in turn could allow for more species to coexist by attenuating competition. There is thus a potential positive feedback loop between local and regional heterogeneity and species diversity (Nathan et al., 2013).

Spatial heterogeneity is particularly relevant for coexistence of sessile organisms like plants (Bolker et al., 2003), and its effect on plant performance can vary according to the life history of the individuals as well as to the particular spatial scale considered. For instance, species colonization in Mediterranean forests has been found to depend on the identity of the dominant species at regional scales during the seed-seedling transition, while it was found to depend on local heterogeneity once seedlings had emerged (Granda et al., 2014). Further, the role of spatial heterogeneity can be strong when coexistence is quantified at scales larger than those perceived by the organisms, e.g., when coexistence of species locally segregated by fine-grained heterogeneity is determined at regional scales. This role of spatial heterogeneity when coexistence is assessed at a coarser grain than that perceived by the organisms has been shown to explain coexistence in the case of microorganisms dwelling in patchy soils (Porter and Rice, 2013). Different scales of heterogeneity can also explain vegetation patterns in Mediterranean ecosystems where dominance of one species at local, patch level is compensated by the co-occurrence of close-by patches dominated by different species. Moreover, in these ecosystems dominated by a few tree species the juveniles have been shown to recruit preferentially in non-conspecific stands, generating dynamic mosaics within a landscape where patches dominated by each species promote species turnover over time (Granda et al., 2012, 2014; Galiano et al., 2013). In addition, metacommunity approaches explicitly link local and regional community dynamics. Gilbert and O'Connor (2013) also highlighted that the metacommunity theory allows scaling up from community-level processes to regional patterns of species distribution and dynamics. Despite their potential for exploring the influence of regional processes, such as dispersal and habitat configuration, on local abundances and occurrences few studies incorporate metacommunity dynamics into a global change framework (Gilbert and O'Connor, 2013) due to the challenge of determining the effects of global change on processes at different scales and to account for their synergy (O'Connor et al., 2012). However, metacommunity models can appropriately guide research on how climate change alters specific local and regional processes and the feedbacks between them determining coexistence (Anderson et al., 2015). In turn, empirical research can identify important gaps in metacommunity approaches (Gilbert and O'Connor, 2013).

Equally important for the maintenance of coexistence is the heterogeneity in time, with an influence on natural communities also variable depending upon the temporal scales. Temporal fluctuations can stabilize coexistence via storage effect (Chesson, 2000), when inter-annual variation in climate or resource availability favors alternatively one group of species over the others (e.g., Zavaleta et al., 2003). Not only inter-annual but also seasonal variability contributes to fluctuating resources that increase the number of coexisting species in different systems (Angert et al., 2009; Shimadzu et al., 2013). Oscillations at the population level can further be a consequence of species interactions with shared resources (i.e., endogenous compensatory dynamics, González and Loreau, 2009), when the species with a saturating growth response generates cycles of the resource. As a result, community dynamics are ensured by both species interactions and different responses to the fluctuating environment.

Disturbance in space and time is important for species coexistence in environments that are relatively homogeneous so it breaks at least temporarily this homogeneity. Such a disturbance regime becomes key for competition-colonization trade-offs (Cadotte, 2007). These trade-offs are the basis for the intermediate disturbance hypothesis, which states that diversity of competing species is maximized at intermediate frequencies or intensities of disturbance or environmental change (Bongers et al., 2009). However, and despite the abundant and interesting research yielded with this hypothesis, a revision of its current theoretical and empirical foundations suggests that it should be abandoned (Fox, 2013). Empirical studies only rarely find the predicted humped diversity-disturbance relationship and the three theoretical mechanisms claimed to produce this relationship are logically invalid (Fox, 2013). Originally created to explain patterns of diversity in tropical forests, its explanatory value is poor even in this ecosystem as shown in an extensive review (Bongers et al., 2009). While diversity did peak at intermediate disturbance levels little diversity variation could be explained outside dry forests since disturbance had less influence on species richness patterns in wet tropical rain forests than typically assumed (Bongers et al., 2009).

Two fundamental drivers of environmental change for plant communities are long-term increases in soil resource availability and grazing pressure (Adler et al., 2001; Laliberte et al., 2013). These changes are expected to produce profound changes in diversity and species composition, and one expects that in general they reduce diversity by exclusion. For those species that coexist thanks to heterogeneous environments, an increase in resource supply can homogenize differences between patches. With a more homogenized environment the likelihood of coexistence is smaller because this tends to favor the species that can better exploit a single environment. For instance, Southon et al. (2013) showed that across the UK, nitrogen deposition is reducing diversity in the heathlands with a few species dominating across regions. Similar results were obtained in a manipulative experiment of a Californian grassland by Zavaleta et al. (2003), where a homogenization of the environment caused by increases in nitrogen deposition decreased the number of coexisting species at patches that were not subjected to any degree of disturbance. Similar losses of diversity can occur when the degree of disturbance is too high, because only a few species will be able to survive in such stressful environments, as it is occurring with the loss of plant, bird, and mammal diversity in intensified rural landscapes (Flynn et al., 2009).

Another functionally important aspect of heterogeneity is the increased frequency and intensity of extreme climatic events caused by climate change. These perturbations are leading to species-specific mortality, changing competitive ability differences among species, reducing the abundance of the dominant species, and, therefore, changing the long-term population and community trends (Thibault and Brown, 2008). Holmgren et al. (2006) showed strong cascade effects from species responses to community-level changes in arid and semi-arid ecosystems worldwide after changes associated to El Niño Southern Oscillation (ENSO). Limited capacity of native communities to maintain their structure and function after extreme climatic events has been shown to favor the invasion process (Diez et al., 2012), and changes in the dominance of native species within communities due to different growth responses and recovery patterns during and after extreme droughts have also been suggested (Cavin et al., 2013; Granda et al., 2013).

All drivers of global change are expected to exert an important effect on coexistence mechanisms and, therefore, to change the outcome of species interactions (**Figure 4**). Anthropogenic environmental and climatic changes for example are dramatically varying the resource supply at multiple spatial and temporal scales (Matesanz and Valladares, 2014; Valladares et al., 2014a). This variation is important because the stability of the resources affects species' abilities to capture them (Nathan et al., 2013; Parepa et al., 2013). Overall, this variation is leading to extinction rates that are significantly higher than what would be expected from the fossil record (Bálint et al., 2011; Barnosky et al., 2011). However, increased diversity is also being observed at global scales in certain ecosystems such as alpine grasslands (Cavieres et al., 2014).

BIOLOGICAL INVASIONS, A COEXISTENCE LESSON IN A CHANGING WORLD

Human activity is transporting, either voluntarily or involuntarily, thousands of species through long distance and although many species fail to establish, some species become new elements of the local biota (Hulme, 2009). Coupled to the global phenomena of biological invasions, a whole body of literature has emerged in ecology to understand the mechanisms by which invasions occur (reviewed in Catford et al., 2009). Biological invasions therefore represent a good example to relate recent advances in coexistence theory to the effect of global change on natural ecosystems, as most of the factors driving invasion can be better understood within a framework of niche and fitness differences. MacDougall et al. (2009) have shown that the mechanisms driving invasion and the maintenance of species diversity are two sides of the same coin. Invasion and exclusion of the native community occur when the fitness advantage of the exotic species overwhelms the stabilizing niche differences with the resident community. Invasion and coexistence with the native residents occur when exotic species are able to enter into the system at low relative abundance because of their niche differences with respect to the native residents but these niche differences prevent them from excluding native species while becoming abundant (MacDougall et al., 2009; **Figure 3**).

Understanding whether invaders benefit from either fitness differences or niche differences, or both is crucial if we aim to control and eliminate invasive species, a common target of restoration and conservation programs. Prior conceptual and experimental work using trait-based approaches has argued for the concept of limiting similarity to accomplish successful restoration actions (Emery, 2007; Funk et al., 2008). The

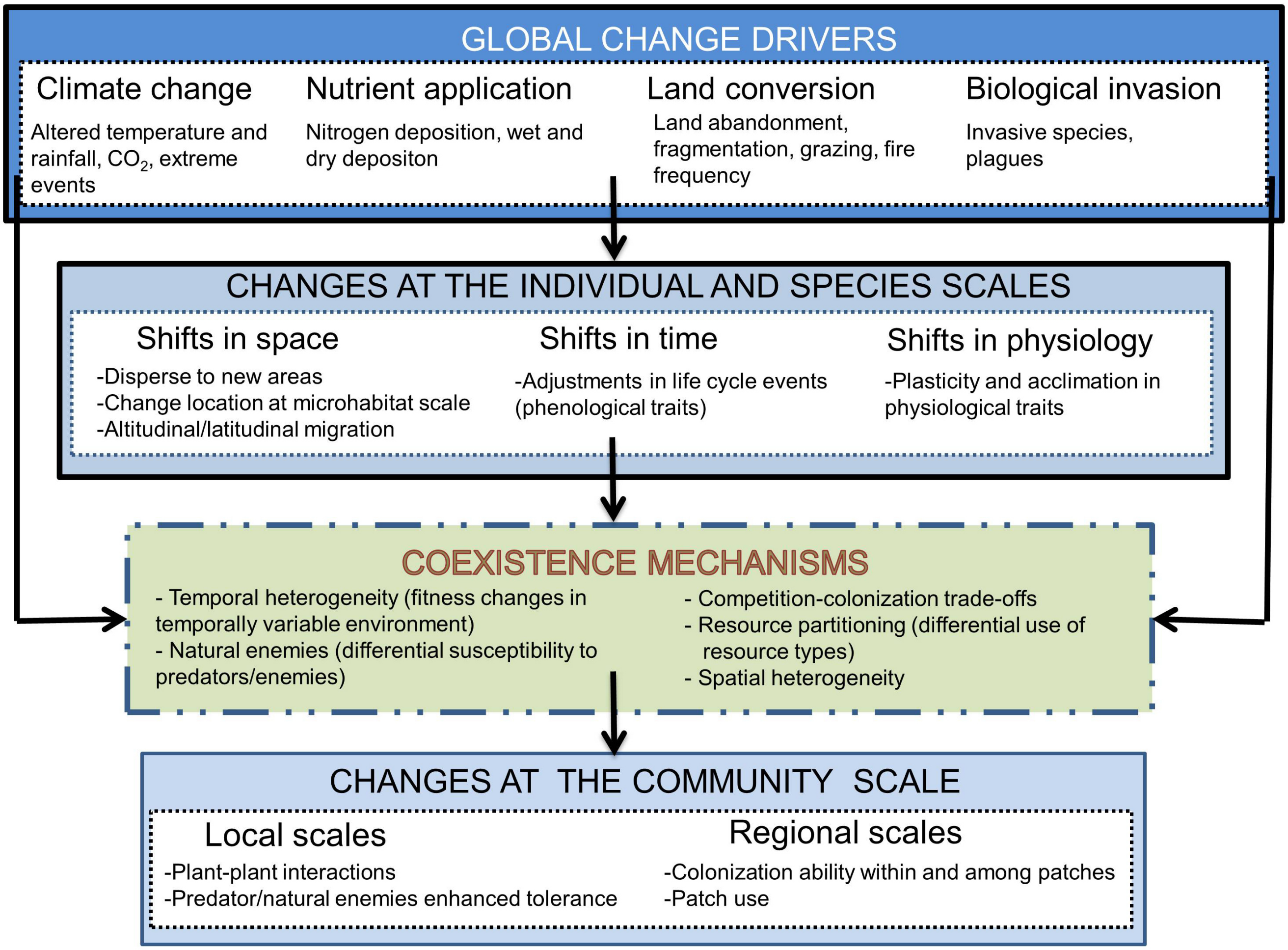

FIGURE 4 | Global change drivers affect coexistence mechanisms in a number of ways, at various levels of biological organization (from individuals to species) and at various spatial and temporal scales. Individual fast responses to environmental change co-occur with alterations in species interactions, resource use and many other changes that interactively affect species coexistence. Changes observed at the community level are thus resulting from the direct effect of global change drivers on both coexistence mechanisms and individual species responses to changes in these drivers.

underlying idea is that functional traits reflect species' niche. Nevertheless, this approach can be limited for the two following reasons. First, native species may not possess the functional characteristics needed to fill the same niche as the invaders. Second, functional traits can reflect both species niche and fitness. In this latter case, using native species with similar characteristics to those of the invader may not turn into the result desired. A clear example focusing again on phenology illustrates this problematic issue. In a California grassland, Godoy and Levine (2014) have found that differences in phenology promote both stabilizing niche differences between annual plant competitors and fitness differences between them. Fitness advantages were greater on later phenology species. Importantly, the fitness advantage of later phenology species overwhelmed the stabilizing effect of phenological offset competitors allowing later invaders to outcompete earlier native communities and native residents to outcompete earlier-phenology invaders (Fridley, 2012). Overall, these results highlight two important messages. First, by focusing only on functionally dissimilar native communities, invasive species with early phenology can be eliminated from the system. Second, some aims of restoration cannot be accomplished. In this example, later invasive annual species cannot be repelled with native annual communities. Perhaps, shrub and tree encroachment could eliminate these later invasive species by shading, which will probably reduce their fitness, but this action is in conflict with the maintenance of a grassland system.

Different drivers of global change can modify niche and fitness differences between invaders and resident communities, and hence modulate the impact of biological invasions (**Table 1**). For instance, climate change can increase invader's population size presumably due to a relative increase in fitness with respect to the native community during periods of climatic amelioration such as increasing temperatures for thermophilous plants (Walther et al., 2002). Climate change, through extreme events such as heat waves, hurricanes, flood, and drought is also expected to promote invasion success (Diez et al., 2012). In general, extreme climatic events produce simultaneously a reduction of the fitness of the native residents and an increase of the fitness of the invaders thanks to a resource pulse. This combination occurs when the stress tolerance of invaders to abiotic factors is higher (Diez et al., 2012; Gioria and Osborne, 2014). For example, non-native vines benefited more than native vines from the full-exposed sun conditions derived from wind-driven tree canopy loss after Hurricane Andrew in Florida in 1992 (Horvitz et al., 1998; see Diez et al., 2012, for other examples).

New niche opportunities favoring invasion come often from anthropogenic changes (Shea and Chesson, 2002), which are ubiquitous components of global change. In general, invasive species maintain self-sustaining populations and disperse through disturbed habitats such as roadsides, railways, human-modified rivers and abandoned cultures that become semi-natural grasslands. The idea that the invader's niche is linked to perturbation, is common (Lake and Leishman, 2004; Pauchard and Alaback, 2004) to the extent that invaders are seen as passengers more than drivers of the habitat changes (MacDougall and Turkington, 2005). The limitation to native species for exploiting these new niches created by anthropogenic changes can come from different functional, ecological and evolutionary sources (Matesanz and Valladares, 2014). For example, water irrigation is creating a new niche in Spanish Mediterranean ecosystems with minimized drought during summer (Godoy et al., 2009). Native species are not able to exploit this niche because of their evolutionary constraints to display mostly a spring phenology. However, invasive species that evolved in tropical environments display summer phenology matching the time frame of resource availability (Godoy et al., 2009). This fact can increase the overall number of species that can be found in a particular ecosystem (Knops et al., 1999), because exotic species do not produce any harm to the native community, but also increases the risk of invasion since rapid evolution to more drought adapted phenotypes can occur easily.

IMPACTS OF GLOBAL CHANGE ON SPECIES COEXISTENCE IN PAUCI-SPECIFIC SYSTEMS: THE CASE OF MEDITERRANEAN FORESTS

Despite being within a biodiversity hotspot, Mediterranean forests are typically dominated by only two-three tree species, particularly in dry, continental areas (di Pasquale et al., 2004). Empirical studies aimed at characterizing mechanisms of species coexistence in Mediterranean forests are scarce. These generally include: (i) differential species responses to environmental stress, (ii) dispersal patterns and, (iii) spatial heterogeneity, which, coupled with facilitation, are recognized as the main mechanisms promoting coexistence (Gómez-Aparicio, 2008; Granda et al., 2012; Pérez-Ramos et al., 2012a, Galiano et al., 2013). Pérez-Ramos et al. (2012b) found within and among species differences through plant ontogeny arising from species differential responses to microhabitat heterogeneity and seed size variation in a mixed-oak forest of southern Spain, further confirmed by Granda et al. (2014) in continental Mediterranean forests. Galiano et al. (2013) also focused on regeneration patterns of oak species in a pine-dominated forest of north-east Spain, where pine mortality was not compensated by its regeneration, suggesting vegetation shifts to oak-dominated forests if the intensity and frequency of extreme droughts keep increasing. In addition to these and other studies addressing coexistence, we suggest that more research is needed to improve our understanding of the specific mechanisms involved, such as those common in species rich ecosystems (i.e., tropical forests) that have been rarely identified in pauci-specific ones (i.e., Mediterranean forests where a few engineering species dominate the canopy). We suggest that negative density dependent processes, including predation, herbivory or pathogen infection could also modulate coexistence in Mediterranean ecosystems by promoting the recruitment away from parent trees and freeing potential colonization areas for other species (Granda et al., 2014). So far, non-random patterns of pathogen infection (predictable by both abiotic and, particularly, biotic factors as tree and shrub species presence) and their role in plant communities have been described in southern Spain (Gómez-Aparicio et al., 2012). However, further research is needed to test whether negative density dependence could promote species coexistence in the Mediterranean region if, for example, infection of the most common species favors the establishment of other species in accordance with the Janzen–Connell hypothesis (Janzen, 1970; Connell, 1971). Moreover, the alteration of these coexistence mechanisms under ongoing global change should be better described to be able to predict future directions in forest dynamics.

Despite the loss of diversity in rich ecosystems being a crucial concern for ecologists and conservationists, the ecosystems that are perhaps more endangered by global change drivers are those containing a low number of species that contribute significantly to its functioning and productivity.

Recent studies have highlighted that the resilience of a system, (i.e., the ability of a community to respond to global changes) depends on the functional diversity of a community rather than its species richness (Diaz et al., 2007). In pauci-specific ecosystems, species loss may have serious consequences for the functional diversity, collapsing the system when the species lost cannot be replaced by another species with similar function. In other words, the limited functional redundancy that is mathematically possible in a pauci-specific ecosystem makes them more vulnerable to species loss at least from a probabilistic point of view. This is the case of many Mediterranean forests, where coexisting species tend to present strong dissimilarities in their traits. Instead, other ecosystems with a higher number of species may show similar functional diversity, indicating that trait values among species are also similar. In these sites, functional redundancy may buffer against the impact of climate change on the local species pool as shown by Gallagher et al. (2012).

Most drivers of global change such as increased aridity, pollution, land use change and increased fire risk, all of them already exerting great pressures on Mediterranean ecosystems (Doblas-Miranda et al., 2014; Valladares et al., 2014a), are presumably going to reduce species fitness up to the point to limit their survival under these new conditions (Matesanz and Valladares, 2014). At least two scenarios emerge as alternatives to the simplistic expectation of species gradual extinction under such increase of environmental pressure: (i) coexistence is maintained by changes in species interactions (increased role of facilitation, complex multi-species interactions reinforced), which can buffer the pressure, and (ii) within species functional variability could compensate for the limited number of species making up the community. Knowledge on factors influencing the occurrence of these two alternatives and on their implications is still very limited to assess their potential for counteracting the negative impacts expected from the increased environmental pressure.

CONCLUSION

There is an urgent need to understand how different drivers of global change differentially but simultaneously impact ecosystems and which are the corresponding magnitude and direction of the changes in species interactions and coexistence. Recent developments of ecological theories are improving the forecast of these changes but more empirical data are needed for a solid theory of the mechanisms driving species coexistence.

There are three main empirical approaches to the study of community assembly: experimental manipulations of the abiotic or biotic environment, assessments of trait-phylogeny-environment relationships, and quantification of frequency-dependent selection and population growth. Each approach alone is not strong enough to reveal which niche axes and which traits determine the outcome of competition, the extent of facilitation and the eventual structure and dynamics of the community. Thus, only the combination of these three approaches can significantly contribute both to conceptual ecology and to guidelines for ecosystem management under global change (HilleRisLambers et al., 2012). Nonetheless, the combination of the three in a single research project requires an enormous effort that sometimes is unjustified. The degree of resolution would depend on the research aim. For instance, if the question is related to how species are precisely responding to a combination of different global change drivers (e.g., an increase in precipitation or aridity, an increase in nitrogen deposition, or an increase in grazing) then to study how these drivers affect species fitness could be enough. However, if the question relates to how specific species responses translate to community dynamics, then it is also necessary to study niche differences among species to know the outcome of species interactions. While the amazing richness of ecosystems like tropical forests have attracted fruitful research and theories on species coexistence, there is much that can be learnt from pauci-specific communities where the value of each single species is large and where the functional variability within each species becomes key to understand species interactions and eventual community responses to global change. In both research and conservation activities, we have to move from species coexistence to the coexistence of genotypes, paying more attention to the functional variability existing within each species.

AUTHOR CONTRIBUTIONS

All authors jointly developed the concept of this paper, contributed with ideas and information and wrote the manuscript.

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Abiotic and biotic controls on local spatial distribution and performance of *Boechera stricta*

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This study investigates the relative influence of biotic and abiotic factors on community dynamics using an integrated approach and highlights the influence of space on genotypic and phenotypic traits in plant community structure. We examined the relative influence of topography, environment, spatial distance, and intra- and interspecific interactions on spatial distribution and performance of *Boechera stricta* (rockcress), a close perennial relative of model plant *Arabidopsis*. First, using Bayesian kriging, we mapped the topography and environmental gradients and explored the spatial distribution of naturally occurring rockcress plants and two neighbors, *Taraxacum officinale* (dandelion) and *Solidago missouriensis* (goldenrod) found in close proximity within a typical diverse meadow community across topographic and environmental gradients. We then evaluated direct and indirect relationships among variables using Mantel path analysis and developed a network displaying abiotic and biotic interactions in this community. We found significant spatial autocorrelation among rockcress individuals, either because of common microhabitats as displayed by high density of individuals at lower elevation and high soil moisture area, or limited dispersal as shown by significant spatial autocorrelation of naturally occurring inbred lines, or a combination of both. Goldenrod and dandelion density around rockcress does not show any direct relationship with rockcress fecundity, possibly due to spatial segregation of resources. However, dandelion density around rockcress shows an indirect negative influence on rockcress fecundity via herbivory, indicating interspecific competition. Overall, we suggest that common microhabitat preference and limited dispersal are the main drivers for spatial distribution. However, intra-specific interactions and insect herbivory are the main drivers of rockcress performance in the meadow community.

Keywords: Bayesian kriging, competition, correlogram, Mantel tests, path analysis, spatial interaction, spatial pattern

INTRODUCTION

Spatial patterns are a crucial, but often overlooked, component to understanding the factors and processes that structure plant communities (Levin, 1992; Jeltsch et al., 1999; McIntire and Fajardo, 2009). Spatial distribution and performance (i.e., growth and reproduction) of any plant species depends on the ability to cope with environment, most notably topography, soil properties, and moisture availability (Goslee et al., 2005), intraspecific genotype variation of plant species (Crutsinger et al., 2006; Lankau and Strauss, 2007), intra- and interspecific plant-plant interactions (Callaway and Walker, 1997; Holzapfel and Mahall, 1999; Pugnaire and Luque, 2001; Brooker et al., 2008; Genung et al., 2012), and insect herbivory (Marquis, 1992; Bloom et al., 2003; Becerra, 2007). Investigating the relative importance of these controlling factors is critical to understanding the underlying processes that structure plant communities. This study describes the first application of an integrated approach using molecular, ecological, and statistical tools to quantify the relative influence of biotic and abiotic factors on the spatial distribution and performance of *Boechera stricta* (rockcress), an emerging ecological model plant species (Rushworth et al., 2011). Such species show great promise for understanding genetic controls on ecologically important traits (Song and Mitchell-Olds, 2011) and provide opportunity to explore underlying mechanisms in natural populations, compared to artificial experimental settings. Rockcress is widely distributed in the western United States (Song and Mitchell-Olds, 2011) and shows local adaptations in diverse ecological habitats (Mitchell-Olds, 2001; Knight et al., 2006; Song et al., 2006), making it an ideal species to study the biotic and abiotic controls on species distribution and growth in natural settings.

Combinations of complementary mathematical and statistical techniques have been used in recent studies to investigate links between observed spatial patterns and underlying ecological processes [e.g., process models and geostatistics (Loranty et al., 2008); multiple ordination and geostatistics (Wagner, 2003); Structural Equation Modeling and Bayesian statistics (Arhonditsis et al., 2006); mixed models and Bayesian kriging

(Smithwick et al., 2012); and Mantel tests and path analysis (Goslee et al., 2005)]. The integration of these tools can provide information about spatial structure of variables and potential underlying ecological processes. Here, we integrated Bayesian kriging (Diggle et al., 1998; Diggle and Ribeiro, 2002), a novel data-model fusion approach for spatial interpolation of topography and environmental data across the local landscape, and Mantel path analysis (Mantel, 1967; Leduc et al., 1992; Goslee et al., 2005) to infer the relative importance of microhabitat preference, limited dispersal, competition, and herbivory in determining the spatial distribution and performance on a local scale.

First, we examined the spatial distribution of rockcress individuals and the density of inter-specific neighbors across the topography of the local landscape, which included gradients of soil moisture, vapor pressure deficit, and topology (measured as elevation). Second, we determined the spatial structure of environmental and intra- and interspecific variables (see Methods for detailed description of these variables) using piecewise Mantel correlograms (Goslee and Urban, 2007). The Mantel correlogram is particularly useful for studying ecological patterns in count data because it provides the spatial information in terms of distance apart rather than geographical location using dissimilarity-based analysis (Urban et al., 2002). Third, we evaluated plausible hypotheses on underlying processes using Mantel path analysis in the presence and absence of space (Goslee et al., 2005). We asked: (1) what governs the spatial distribution and performance of rockcress on a local scale? and (2) what is the relative importance of factors governing the spatial distribution and performance at this scale?

MATERIALS AND METHODS

STUDY SITE

We studied spatial distribution and performance of rockcress [*Boechera stricta* (previously *Arabis drummondii* (Al-Shehbaz, 2003)] plants occurring within a conspicuous highly populated local area in the northern Black Hills, South Dakota, USA, (Lat: 44.403611, Long: −103.938403, elevation 1365 m) during the summer of 2004. Rockcress plants grew within a 40 × 50 m meadow area surrounded by ponderosa pine (*Pinus ponderosa*), aspen (*Populus tremuloides*), birch (*Betula spp.*), and burr oak (*Quercus macrocarpa*). Other common plant species that inhabited the meadow were goldenrod (*Solidago missouriensis*), dandelion (*Taraxacum officinale*), mouse-ear chickweed (*Cerastium vulgatum*), snowberry (*Symphoricarpos albus*), and sedge (*Carex spp.*). The relief between the highest and the lowest measured point across the study area was 9.55 m.

ENVIRONMENTAL DATA

A hand-held probe (Theta Probe, Delta-T Devices) was used to measure surface (0–6 cm) soil moisture and a hand-held sling psychrometer (Bacharach 12-7012; Bacharach, Inc., New Kensington, PA) was used for vapor pressure deficit. These measurements were taken at 71 randomly selected locations once to represent the spatial gradient of environmental variables across the meadow.

SAMPLING

Every rockcress individual ($n = 234$) in the study area was geolocated and sampled for growth (diameter of basal rosette, *rosette diameter*) and reproductive fitness (number of reproductive stalks, *stalk number*; reproductive stalk diameter, *stalk diameter*; and number of fruits, *fruit number*). In addition, amount of herbivore damage (percent area consumed) on basal rosette leaves (*rosette herbivory*) and reproductive stalk leaves (*stalk herbivory*) were recorded. Herbivory was quantified for each plant by counting the number of holes (≥1 mm) that the flea beetles chewed on the leaves of basal rosette and reproductive stalks. To determine the influence of neighboring species on rockcress growth and performance two plant species, goldenrod and dandelion were selected due to their abundance in the landscape in close proximity to rockcress as compared to other species. The number of goldenrod (*goldenrod density*) and dandelion (*dandelion density*) plants within a 15 cm radius of each rockcress plant was recorded. The 15 cm radius was an appropriate distance because of the small size and high density of the meadow plants.

Additionally, to distinguish between environmental and genetic causes for spatial patterns, we genotyped 142 randomly selected rockcress plants using seven polymorphic microsatellite loci and STRUCTURE software (Pritchard et al., 2000), available at http://pritchardlab.stanford.edu/structure.html. Briefly, this technique identifies groups of relatives, which in this case are self-fertilizing (inbred) lineages (hereafter, *line*), or in other words, overlapping-generation dependents from common ancestors. Please see detailed description of this technique in Siemens et al. (2009).

STATISTICAL PROCEDURES

Bayesian kriging

Maps of environmental variables were generated using spatial interpolation in a Bayesian framework. A fully probabilistic Gaussian spatial process model (Diggle et al., 1998; Diggle and Ribeiro, 2002) was used for Bayesian kriging, which assumes that conditional on a Gaussian underlying process, S the observed data, Yi: $i = 1, \ldots n$ are independent with a distribution in the exponential family. A brief summary of the modeling approach is given below; detailed information can be found in Diggle and Ribeiro (2002). The model can be expressed in a hierarchical framework as follows:

$$\begin{aligned} &\text{Level 1}: Yi|S \sim N(\beta(xi) + S(xi), \tau^2)\\ &\text{Level 2}: S(xi) \sim N(0, \sigma^2 R(h; \Phi))\\ &\text{Level 3}: \text{prior}(\beta, \sigma^2, \Phi, \tau^2)\end{aligned} \tag{1}$$

The first level describes a spatial linear trend (β = trend parameter) based on spatially referenced explanatory variables. τ^2 (nugget) represents measurement variability and/or spatial variation below the sampling grain. The second level describes a stationary Gaussian spatial process ($S(xi)$) with mean = 0, variance = σ^2 and correlation function $R(h; \Phi)$, where Φ is correlation parameter (range of spatial autocorrelation = 3Φ) and h is lag distance (vector distance between two locations), and the third level specifies the prior for the model parameters. We chose an exponential correlation function to quantify spatial autocorrelation:

$$R(h; \Phi) = \exp(-h/\Phi) \tag{2}$$

The mean and variance of topography and environmental data were estimated at individual locations from the predictive distribution using the *krige.bayes* function of geoR library (Ribeiro and Diggle, 2001) in R version 2.15.2 (R Development Core Team, 2012). This algorithm samples a parameter value from a discrete posterior distribution computed from joint distribution of parameters and priors. We assumed a constant trend mean model and chose a sensible interval of values for each parameter considering the study site to generate a multidimensional parameter [Φ, σ^2, and $\tau^2.rel$ (relative nugget $= \tau^2/\sigma^2$)] grid. Please refer to Appendix for exact intervals for individual parameters. Flat priors (see **Figures A1A–C** for an example of prior and posterior distributions) were chosen for Φ, and $\tau^2.rel$, and a reciprocal prior for σ^2. The sampled parameter value is then attached to [β |Y, Φ, σ^2, $\tau^2.rel$] and a realization is obtained from the predictive distribution at an unsampled location. A large sample size was generated by repeating this process several times which allowed a stable estimation of the underlying distribution. The mean and the variance of the predictive distribution were

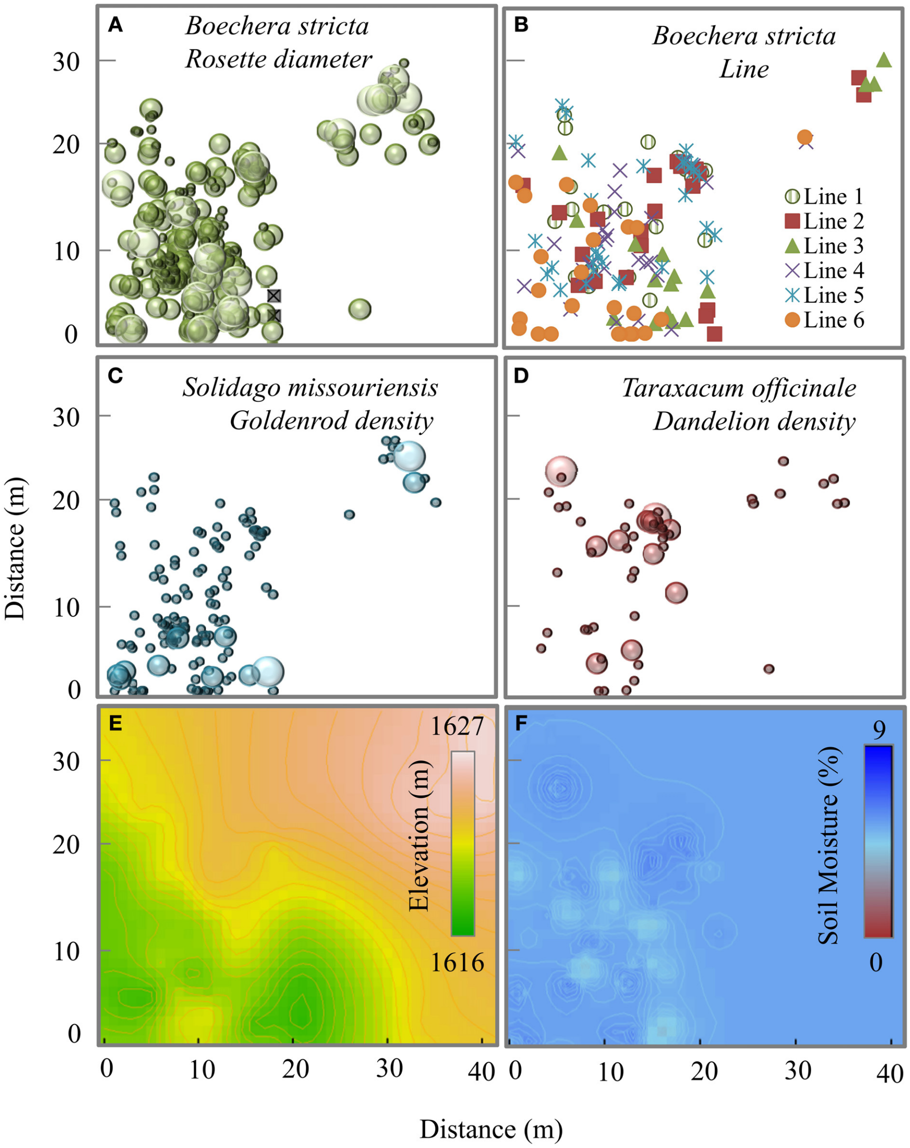

FIGURE 1 | Map of spatial distribution of (A) *Boechera stricta* plants, each circle indicates the presence of one plant and diameter of the circles corresponds to the *rosette diameter* classes from 1–24, 25–45, and 46–60 cm. *Rosette diameter* zero is marked with an *x* indicating previously present plant which is currently dead; **(B)** a random sample (142 individuals) of *B. sticta* plants showing 6 putative naturally occurring near-inbred lines (*line* 1–6) **(C,D)** number of *Solidago* and *Taraxacum* plants within a 15 cm radius around each *B. stricta* plant, diameter of the circles correspond to the number classes 1–20, 21–40, and 41–60. **(E,F)** Kriged maps of elevation and soil moisture for the study site.

computed at unsampled spatial locations using 100,000 posterior draws. Leave-One-Out cross-validation (**Figure A1** in Appendix) was used for model validation. The maps of environmental variables were used to obtain data at individual plant's location to conduct the Mantel correlation analysis.

The piecewise Mantel correlogram and Mantel test

A correlogram in this context is a plot of the spatial autocorrelation with lag distance, and the Mantel test can be used to test the significance of the correlations. A simple Mantel test (Mantel, 1967; Goslee and Urban, 2007) was performed using Euclidean distance to explore variation in spatial structure of topography (local vertical relief measured as elevation), environmental drivers (soil moisture and vapor pressure deficit), fitness parameters [growth (*rosette diameter*) and reproduction (*stalk numbers*, *stalk diameter*, *fruit number*), and *line*] of rockcress, density of neighboring plants (*goldenrod density* and *dandelion density*), and herbivory on rockcress leaves (*rosette herbivory* and *stalk herbivory*). Data were standardized before calculating Euclidean distances (as recommended by Goslee, 2010) and the error was computed using 10,000 permutations. Autocorrelation range was determined by looking at the significance value ($P = 0.05$) of the individual bins of lag distance at every 2.5 m in the correlograms. A pairwise correlation between all the variables was determined using simple Mantel test that guided the path analysis.

Mantel path analysis

Path analysis is a regression technique to explore causal connections among relevant factors. We used Mantel path analysis to quantify direct and indirect influence of various biotic and abiotic factors on spatial distribution and performance of rockcress population. Partial Mantel tests were conducted to evaluate the plausible hypotheses (generated by combining the information from correlograms and pairwise Mantel tests) on underlying ecological processes by looking at the relationship between two variables and keeping all other variables constant. For example, given the *a priori* hypothesis that A effects B and B effects C, the partial Mantel test can be used to test if C effects A in the absence of B, $C\sim A|B$. If A and/or C displayed significant spatial autocorrelation then space was added as an additional partial, $C\sim A|\text{Space} + B$, in the partial Mantel test (See **Table A1** in Appendix for all the hypotheses). The direction of relationships was predetermined based on the common ecological knowledge. For instance, local relief influences soil moisture and not the other way. Similarly, the number of reproductive stalks influences the number of seeds and not *vice versa*. When the direction could not be determined using this approach then it was left as a simple correlation between two variables. The R package "ecodist" (Goslee and Urban, 2007) was used to calculate the Mantel correlogram and conduct Mantel path analysis.

RESULTS

WHAT GOVERNS THE SPATIAL DISTRIBUTION AND PERFORMANCE OF A ROCKCRESS POPULATION?

Microhabitat preference

Rockcress plants were not evenly distributed across the local landscape, occurring at highest densities at the lower end of the N-facing slope and at relatively high soil moisture levels (**Figures 1A–F**). All variables displayed significant spatial autocorrelation except soil moisture (**Figures 2A–C**). Elevation displayed significant spatial autocorrelation (**Figure 2A**) and a negative relationship with soil moisture (Mantel tests, $P = 0.05$, **Figure 3A**), which was also present after accounting for the spatial structure (partial Mantel tests, **Figure 4**). In general, rockcress size and reproduction showed a negative relationship with elevation, a positive relationship between soil moisture and no relationship with vapor pressure deficit. Thus, rockcress occurred more often in lower, moist areas and in these areas growth and reproduction were also higher. These patterns were reflected in correlograms showing significant ($P \leq 0.05$) spatial autocorrelation among rockcress individuals (**Figure 2B**) and Mantel tests showing significant negative correlation between elevation and

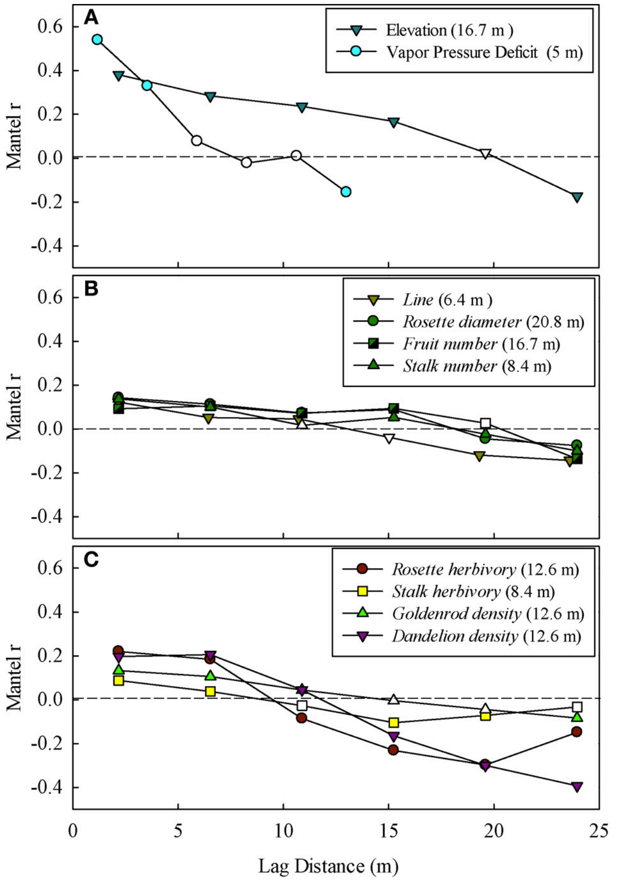

FIGURE 2 | Mantel correlograms showing significant spatial autocorrelation for (A) abiotic environmental factors (Elevation, and Vapor pressure deficit), (B) intra-specific performance and fitness measures (*rosette diameter, stalk number, stalk diameter, fruit number*, and line), and (C) inter-specific neighbors and herbivory (*goldenrod density, dandelion density, rosette herbivory*, and *stalk herbivory*). Filled (significant) and open (non-significant) circles represent statistical significance ($P \leq 0.05$) of Mantel correlation coefficients and each point marks the middle point of the respective lag distance bin. The range of autocorrelation for each variable is given next to the legend. Only significant ($P \leq 0.05$) correlograms are shown.

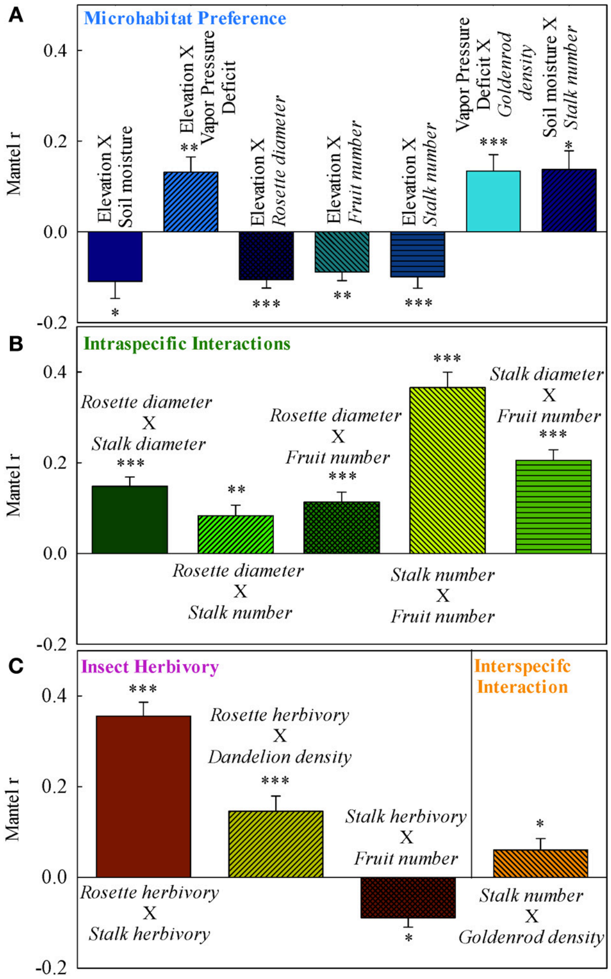

FIGURE 3 | Simple Mantel correlations for (A) environmental controls, (B) intra-specific interactions, and (C) inter-specific interactions. Error bars mark the 90% confidence interval for correlation coefficients. ***$P \leq 0.001$; **$0.001 < P \leq 0.01$; *$0.01 < P \leq 0.05$. Only significant ($P \leq 0.05$) interactions are shown.

rockcress (*rosette diameter, stalk number, and fruit number*) as well as a positive relationship between soil moisture and rockcress (*stalk number*) (**Figure 3A**). The relationship between soil moisture and stalk number was not significant after accounting for space (**Figure 4**). Vapor pressure deficit had no influence on rockcress size or fecundity. Dandelion density around rockcress was highest toward mid-elevations (**Figure 1D**) and no influence of vapor pressure deficit was detected. The density of goldenrod plants around rockcress was positively correlated with high vapor pressure deficit (**Figure 3A**) but this relationship was not significant after accounting for space.

INTRA-SPECIFIC INTERACTIONS

Rockcress growth (*rosette diameter*), reproduction (*stalk number, fruit number*), and line were significantly spatially autocorrelated (**Figure 2B**) with varying ranges from 6.4 m (*line*) to 20.8 m (*rosette diameter*). Genetically similar individuals were found in close proximity of one another (spatially autocorrelated, **Figure 2B**) and *rosette diameter* had positive influence on reproduction with and without space (**Figures 3B**, **4**). Spatial distribution patterns of rockcress had both genetic (**Figure 2B**) and environmental (**Figures 3A**, **4**) components.

INSECT HERBIVORY ON ROCKCRESS

Herbivory on rockcress rosette and reproductive stalk leaves showed significant ($P = 0.05$) spatial autocorrelation [*rosette herbivory* (range = 12.6 m), *stalk herbivory* (range = 8.4 m)] (**Figure 2C**). Spatial cross-correlation (Mantel tests, **Figure 3C**) and cross-correlations after removing multiple correlation and space (Partial Mantel tests, **Figure 4**, **Table A1** in Appendix) showed positive relationship between *rosette herbivory* and *stalk herbivory*. Interspecific neighbor density (*dandelion density*) around rockcress was positively correlated with *rosette herbivory* (**Figures 3C**, **4**), and herbivory on the leaves of reproductive stalks (*stalk herbivory*) was negatively correlated with fruit production (*fruit number*) (**Figures 3C**, **4**), indicating negative impact of *rosette herbivory* on rockcress reproductive performance.

INTER-SPECIFIC INTERACTIONS

Mantel correlograms showed significant ($P = 0.05$) spatial autocorrelation in neighboring densities of goldenrod (range = 12.6 m) and dandelion (range = 12.6 m) (**Figure 2C**). Partial Mantel tests did not indicate any relationship between rockcress and neighboring densities of goldenrod and dandelion.

WHAT IS THE RELATIVE IMPORTANCE OF FACTORS GOVERNING THE SPATIAL DISTRIBUTION AND PERFORMANCE OF A ROCKCRESS POPULATION?

The spatial distribution of rockcress individuals across the landscape was greatly influenced by the spatial distance (significant spatial autocorrelation, **Figure 2B**) and local relief (**Figure 3**). Rockcress plants were generally crowded in N-facing slopes at lower elevations where there is shade by trees (**Figure 1**). Soil moisture and vapor pressure deficit did not show any significant influence on spatial distribution of rockcress plants. Range of autocorrelation for genotypic diversity (*line*) was the lowest among intra-specific traits that indicates greater intra-specific diversity of rockcress plants within short distances. Inter-specific interactions showed some indirect influence on rockcress distribution mediated through increased herbivory (**Figure 4**). Overall space and elevation and has the greatest influence on spatial distribution of rockcress individuals.

Elevation, soil moisture, and vapor pressure deficit did not display any significant influence on rockcress performance. Intra-specific interactions and insect herbivory are the main drivers of rockcress performance. Additionally, indirect influence of inter-specific interactions on rockcress performance were evident (**Figures 3**, **4**).

DISCUSSION

MICROHABITAT PREFERENCE

Spatial autocorrelation for plant performance and genetic variation indicate spatial aggregation among related plants for general fitness, which is most likely a consequence of limited dispersal

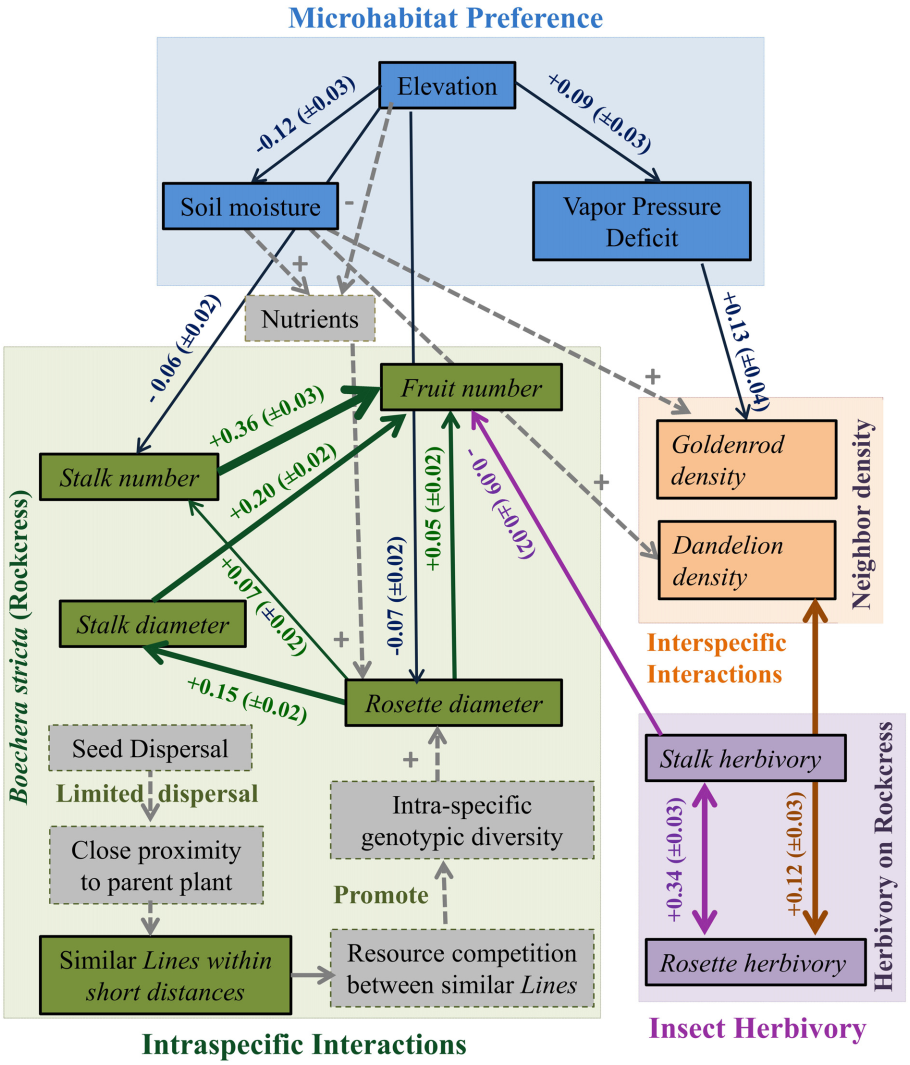

FIGURE 4 | An ecological network of interactions between abiotic environmental factors (Elevation, Vapor pressure deficit, Soil moisture, and Nutrients), intra-specific performance and fitness measures (*rosette diameter, stalk diameter, fruit number, stalk number*, and *line*), and inter-specific neighbors and herbivory (*goldenrod density, dandelion density, rosette herbivory*, and *stalk herbivory*) in a rockcress population. Boxes represent observed variables and arrows represent significant partial correlations. Sign associated with each arrow describes the positive or negative influence. Thickness of arrows is related to the Partial Mantel correlation coefficient associated with each arrow and 90% confidence interval is given in the parentheses. Unidirectional arrows represent positive or negative influence and bidirectional arrows represent simple correlations. The dotted gray boxes display the variables not measured in this study and dotted gray arrows describe the proposed mechanisms to explain the relationships.

and habitat heterogeneity. Facilitation among con-specific plants is a less likely explanation, given inter-plant spacing among rockcress individuals was typically much greater than the reach of canopies or root systems; the nearest neighbors of the rockcress plants were often other species of plants. Genetic similarities decreased at shorter distances (<5 m) than did performance or fitness measures (8–20 m) and landscape attributes (e.g., topography, ~16 m), indicating that clustering of similar sized plants had both genetic and micro-environmental influences. To date, local adaptation in rockcress for defensive traits has been documented on a broad geographic scale (Prasad et al., 2012). We propose that a greater diversity of functional genes could be present in the rockcress population at finer geographical scales due to adaptation to local microenvironments, limited dispersal, and a predominantly self-fertilizing breeding system (Song et al., 2006).

Elevations in the Black Hills (e.g., our study site at about 1700 m) are much lower than elevations where this species (*B. stricta*) of rockcress is usually found in other mountain ranges (about 2500 m) (Mitchell-Olds, personal communication). Probably as a consequence of lower and drier habitats in the Black Hills, we find rockcress on N-facing slopes and in shaded areas. Recent genotyping studies indicate that rockcress in the Black Hills probably originated from lower latitudes in the southern Rockies (Lee and Mitchell-Olds, 2011) where rockcress may be adapted to drier climates. This study was focused on the spatial characteristics and only collected environmental data for 1 day across the meadow. Further work is needed to assess the different spatial and temporal scales at which local adaptation, in particular to dry environments, may occur in this system.

INSECT HERBIVORY ON ROCKCRESS

Insect herbivores are known to reduce the fitness of plants in the wild (Marquis, 1992) and previous studies (Bloom et al., 2003; Prasad et al., 2012) on rockcress have reported a significant negative correlation between rockcress fecundity and herbivory on rosette and reproductive stalk leaves. However, the direct and indirect environmental effects on susceptibility to herbivores are also likely to be important in this system. Based on significant spatial autocorrelation and strong positive correlation of flea beetle herbivory on rosette and reproductive stalk leaves of rockcress, we suggest that plants in close proximity to an infested plant are more prone to flea beetle attack than the plants that are further away. This spatial clustering of herbivory among plants could possibly be due to (1) direct and indirect microhabitat (indirect effects of the environment include effects on host plant resistance through stressful environments and competitors), (2) spatial association of plants within a *line* with similar susceptibilities and resistances, and (3) that flea beetles tend to attract one another. It should be noted that we did not detect a significant correlation between *line* and herbivory, despite the detection of significant genetic variation from common garden experiments among these lines in resistance and glucosinolate toxin production in previous studies (Siemens et al., 2009). Thus, the micro-environmental component to spatial variation in herbivory appears to be more important than genetic variation. Additionally, the positive correlation between *rosette herbivory* and *dandelion density* indicates an environmental component to the susceptibility. Because rockcress and dandelion plants did not often occur in close proximity to one another for any competitive interactions to occur between them, we suggest that increased densities of dandelion may be an indicator of a certain habitat quality that somehow affects rockcress susceptibility. For example, less optimal habitats in this system may stress plants and make them more susceptible to herbivory. Molecular studies on *Arabidopsis* indicate that responses to drought stress may attenuate defense responses to disease and herbivores (Fujita et al., 2006). Additional studies suggest that experimental drought stress in rockcress lowers basal levels of glucosinolates (Alsdurf et al., 2013) and we have observed dramatic increases in herbivory on rockcress plants during severe drought (David H. Siemens, personal observations).

INTER-SPECIFIC INTERACTIONS

Increased densities of dandelion and rockcress may nonetheless indicate increased competitive interactions because the meadow community studied was diverse (∼41 plant species were recorded in 499 micro-communities [100 cm^2] around 499 rockcress individuals in a common garden in this same meadow) and dense (∼70 plants/100 cm^2, $n = 499$) (Siemens and Haugen, 2013). In a common garden experiment in the study area set up across the local range boundary, Siemens and Haugen (2013) found that the decreased performance of rockcress was correlated with the change in community structure. Removal experiments in the field are needed to determine whether the stress that is correlated with community change is caused by competitive interactions (Mulder and Ruess, 1998; Van der Wal et al., 2000). Other species that thrive in similar elevation and soil moisture ranges to rockcress and goldenrod might be expected to be more competitive for space, nutrients, and moisture. However, rockcress is likely to have weaker competitive interactions with other species, like dandelion, that occupy slightly different microhabitats, i.e., relatively higher elevation and drier areas. Goldenrod did not have negative influence on rockcress while dandelion showed positive relationship with rockcress herbivory. Species like dandelion that occupy drier habitats may allocate more resources to roots and thus would be expected to be better below ground competitors. The root-shoot ratio of goldenrod [∼0.4 (Johnson and Biondini, 2001)] makes it a potentially poorer competitor than dandelion [2.5 (Thomas and Bazzaz, 1996)] and rockcress [1.5 (Haugen et al., 2008)]. Our results support previous findings (Eskelinen, 2008) suggesting that positive and negative interactions may vary on target species. However, because of the significant spatial segregation, different habitat preferences, and overall low densities of dandelion and goldenrod within the community it is more likely that the spatial associations and correlated effects on rockcress performance are caused by effects of the entire community, limited dispersal and variation in microhabitats in space and time. Recent studies suggest the importance of intra- and interspecific genotype interaction in structuring the plant community (Crutsinger et al., 2006; Lankau and Strauss, 2007; Genung et al., 2012) and results from this study indicate the spatial distribution has both genetic and environmental components. Overall, this study suggests that common microhabitat preference and limited dispersal are the main drivers for spatial structure of the rockcress population. However, intra-specific interactions and insect herbivory are the main drivers of rockcress performance in the meadow community.

Abiotic and biotic interactions are interconnected strongly in space. The combination of complementary statistical tools (Bayesian kriging, the piecewise Mantel correlogram and Mantel path analysis) enhanced our understanding of the underlying ecological processes of complex spatial interactions and allowed us to dissect direct and indirect effects of biotic and abiotic factors on distribution and performance of a rockcress population. Our attention to spatial distributions and associations expands the possible causations underlying community assembly associations over non-spatial analyses and our results help generate hypotheses for future experimental studies in ecological and evolutionary genomics.

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APPENDIX

Table A1 | Hypotheses derived from correlograms and simple Mantel tests, and tested by using partial Mantel tests.

Hypothesis	Mantelr	*p*-value	90% CI
psm∼ele|space	0.115	**0.02**	0.03
vpd∼ele|space	0.092	**0.02**	0.03
rd∼ele|space	0.068	**0.00**	0.02
fn∼ele|space,rd,rsn	0.029	0.13	0.01
rsn∼ele|space,rd	0.057	**0.04**	0.02
so∼vpd|space	0.128	**0.00**	0.04
fn∼rd|space,rsd,rsn	0.049	**0.04**	0.02
fn∼rsn|space,rd	0.358	**0.00**	0.03
fn∼rsd|space,rd	0.196	**0.00**	0.02
rsd∼rd|space	0.146	**0.00**	0.02
rsn∼rd|space	0.072	**0.02**	0.02
rd∼line|space	−0.004	0.57	0.01
fn∼prh|space	−0.001	0.49	0.02
fn∼psh|space	0.093	**0.01**	0.02
psh∼prh|space	0.342	**0.00**	0.03
prh∼da|space	0.142	**0.00**	0.03
psh∼da|space	0.091	**0.02**	0.03
prh∼da|space,psh	0.119	**0.01**	0.03
psh∼da|space,prh	0.045	0.16	0.03

Bold p-value indicates significant correlation.

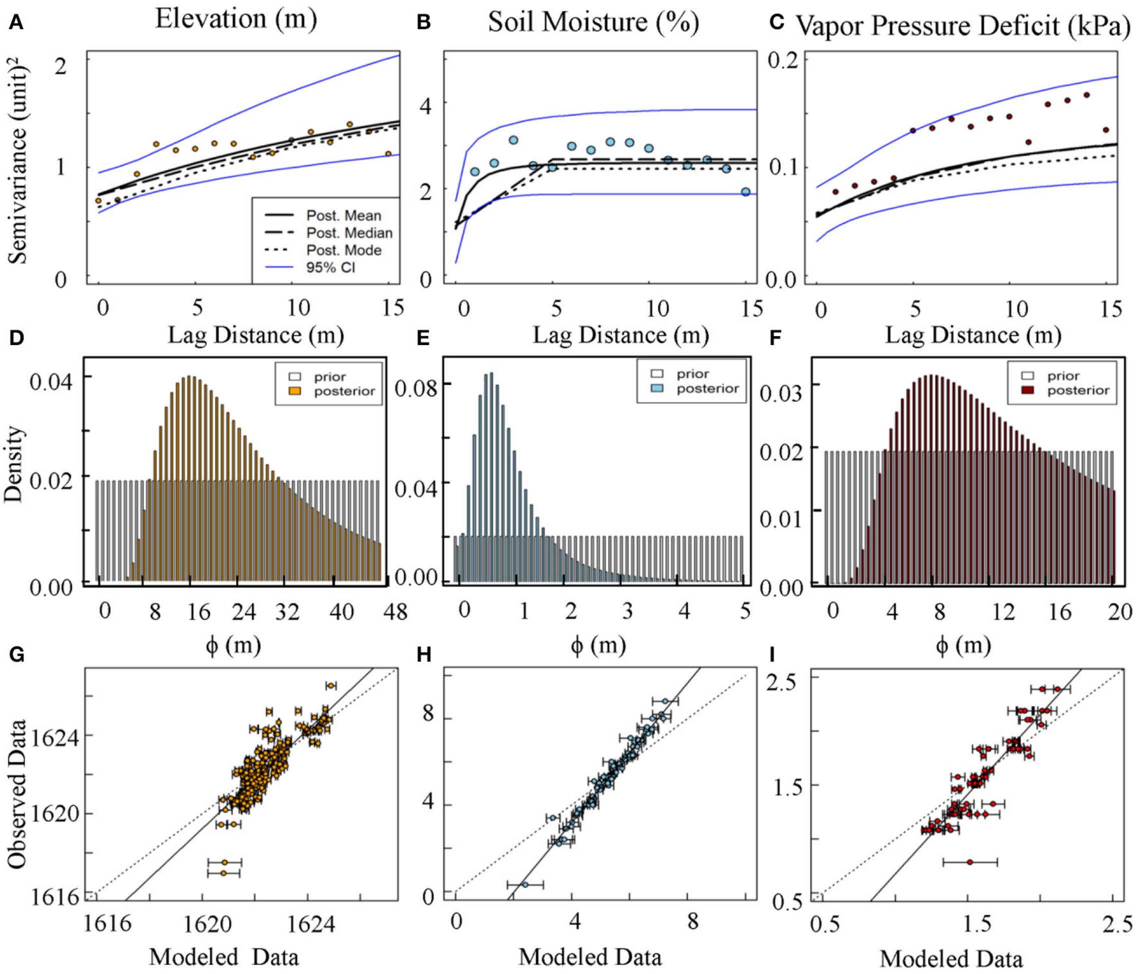

FIGURE A1 | Semivariogams (A–C), prior and posterior distribution of range parameter (range = 3Φ) (D–F), and cross-validation graphs (G–I) associated with elevation, soil moisture, and vapor pressure deficit.

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Stimulating seedling growth in early stages of secondary forest succession: a modeling approach to guide tree liberation

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Excessive growth of non-woody plants and shrubs on degraded lands can strongly hamper tree growth and thus secondary forest succession. A common method to accelerate succession, called liberation, involves opening up the vegetation canopy around young target trees. This can increase growth of target trees by reducing competition for light with neighboring plants. However, liberation has not always had the desired effect, likely due to differences in light requirement between tree species. Here we present a 3D-model, which calculates photosynthetic rate of individual trees in a vegetation stand. It enables us to examine how stature, crown structure, and physiological traits of target trees and characteristics of the surrounding vegetation together determine effects of light on tree growth. The model was applied to a liberation experiment conducted with three pioneer species in a young secondary forest in Vietnam. Species responded differently to the treatment depending on their height, crown structure and their shade-tolerance level. Model simulations revealed practical thresholds over which the tree growth response is heavily influenced by the height and density of surrounding vegetation and gap radius. There were strong correlations between calculated photosynthetic rates and observed growth: the model was well able to predict growth of trees in young forests and the effects of liberation there upon. Thus, our model serves as a useful tool to analyze light competition between young trees and surrounding vegetation and may help assess the potential effect of tree liberation.

Keywords: forest restoration, gap creation, photosynthesis model, light competition, Vietnam

INTRODUCTION

Disturbed or degraded primary forests can recover to a certain extent through secondary succession, which is in essence a continuous replacement of tree species. Native and exotic species that grow after disturbance affect natural forest recovery in different ways (Ortega-Pieck et al., 2011).

The presence of (remnant) native trees and grasses often create a favorable climate for other (pioneer) species to establish and recruit (Carpenter et al., 2004; Muñiz-Castro et al., 2006; Zahawi and Augspurger, 2006; Miao et al., 2013). Once pioneer species have established they can replace the herbaceous layer and create microclimatic conditions in which a more diverse community of later successional species can regenerate (Finegan, 1996; Peña-Claros, 2003; Guevara et al., 2005; Hughes et al., 2012).

However, natural forest recovery after degradation is often slow or stagnates due to excessive growth of exotic shrubs, lianas or grasses (Guariguata and Dupuy, 1997; DeWalt, 2003; Hooper et al., 2004; Davies and Semui, 2006; Esquivel et al., 2008). These species affect the establishment of woody species through competition for a variety of resources including light, water and nutrients (Holl et al., 2000; Parrotta et al., 2002; Hooper et al., 2005; Hoffmann and Haridasan, 2008; Ortega-Pieck et al., 2011). In wet tropical forests especially competition for light seems to play a predominant role in determining the course of succession (Gilbert et al., 2001; King et al., 2005; Selaya and Anten, 2010).

In recent years there has been increased attention for restoration ecology and in particular for management options to accelerate recovery of forests (Parrotta et al., 1997; Holl and Kapelle, 1999; Chazdon, 2013). One often used method to increase (native) tree growth and thus facilitate more rapid succession involves removing the aboveground parts of the vegetation surrounding target trees (Chapman and Chapman, 1999; Fuhr et al., 2001; Dolanc et al., 2003; Duncan and Chapman, 2003). This method, called liberation, can stimulate growth of these individual trees by reducing competition with neighboring plants, especially competition for light. However, liberation has not always had the desired effect (Collet et al., 1998; De Graaf et al., 1999; Otsamo, 2000; Chapman et al., 2002).

Liberation increases light levels around target trees. Studies have shown that light requirements differ among species (Ramos and del Amo, 1992; Montagnini et al., 1997; Dupuy and Chazdon, 2006), even those that are closely related (Korpelainen et al., 1995), and can change with tree age (Davidson et al., 2002). Thus, the effects of increasing light availability through liberation may differ depending on the species-specific physiological traits of the tree being liberated. A better understanding of the interaction between the target species and the surrounding vegetation is needed in order to improve the success of attempts to restore tropical forests (Hardwick et al., 2004). Doing this experimentally however, requires a lot of time, space and money and the outcome is usually restricted to a specific set of species in a specific setting and a limited amount of experimental conditions (for instance gap size) that are created (see Paquette et al., 2006 and references therein).

In this study we present a three-dimensional model (see Supplementary Material) to complement experimental research, which enables us to examine how stature, crown structure, and physiological traits of the target trees and the density and height of the surrounding vegetation in concert determine the whole-plant photosynthesis of target trees. Height, crown dimensions, leaf area, leaf angle distribution, and leaf physiological characteristics of target trees can be varied. The characteristics of the surrounding vegetation (Leaf Area Index, leaf angle distribution, height) can also explicitly be specified and the effects of various types of management practices (e.g., gap creation) can be simulated. This model is used as a first approach to determine the responses of different species to varying levels of release from light competition through liberation. As noted above in moist tropical forests light competition is important in driving early secondary succession and a primary effect of tree liberation is release from this competition.

The model was applied to a dataset from a young natural secondary degraded forest in Vietnam. In South East Asia species of the shrub *Melastoma* grow excessively on degraded lands (Davies and Semui, 2006) and are known to inhibit succession (DeWalt, 2003). Also, large areas of (secondary) forests are converted into grasslands dominated by *Imperata cylindrica* (Werger, 1983; Otsamo et al., 1997), which cause severe competition with emerging trees. Seedlings of pioneer tree species that recruited after a slash and burn treatment were monitored over time (see Van Kuijk et al., 2008). In a 1.5 years old stand half of the studied individuals were liberated of surrounding vegetation. We used model calculations to determine the effect of liberation of individual trees of three woody species in terms of light capture and photosynthesis. The validity of the model was critically tested by comparing predicted tree photosynthetic rates for a given point in time to subsequent growth rates, an approach also used in other studies on light competition (Hikosaka et al., 1999). We also simulated the effects of vegetation LAI and height and gap radius in vegetation removal events, and we predicted the effect of liberating target trees in stands of different successional status.

METHODS

STUDY AREA

The study site is located in the buffer zone of Bach Ma National Park, Thua Thien Hue Province, in central Vietnam (16°10′N 107°50′E). Bach Ma National Park and its buffer zone were established in 1991 with a total area of 43,331 ha. It is the core of the last remaining contiguous forest belt in Vietnam, stretching from the South China Sea to the border with Laos. The area experiences high rainfall, especially from November until February (up to 8000 mm per year). There is no distinct dry season and the vegetation is evergreen (Tran and Ziegler, 2001). After chemical destruction of forests in the war, the study site was used for monoculture plantations of *Acacia mangium* for several decades. Part of the site was left fallow in 1999.

STUDY SPECIES

In November 2004 we applied a slash and burn treatment in a 5-year old forest (the part that was left fallow in 1999) (see Van Kuijk et al., 2008 for details). The woody species that recruited afterwards were monitored over time. For this study the three most abundant recruiting tree species were selected: *Mallotus microcarpus*, *Mallotus paniculatus* and *Macaranga denticulata* (all Euphorbiaceae). Species of *Mallotus* and *Macaranga* are known to regenerate on deforested or degraded lands (Slik et al., 2003; Lee et al., 2005; Toma et al., 2005) and are characteristic of secondary forests in South-East Asia (Steenis, 1965; Primack and Lee, 1991). The role of these pioneer tree species in succession is important as they have the ability to overgrow grass and shrub species that may strongly hamper succession (see also Finegan, 1984, 1996).

MEASUREMENTS

Measurements were performed in April 2006, 1.5 years after the slash and burn treatment (1.5 y/o stand hereafter). Crown allometry of the individual target trees (20 individuals for *Mallotus microcarpus*, 31 for each of *Mallotus paniculatus* and *Macaranga denticulata*) was determined by measuring crown dimensions in four wind directions and from the bottom to the top of the crown. As part of another experiment on the same individuals, biomass allocation, photosynthetic characteristics and nitrogen contents, and data on the vegetation in which they grew, were measured (see Van Kuijk et al., 2008 for detailed methods). A summary of those methods is provided below.

After initial measurements, half of the individuals were selected randomly distributed over the study area to be removed of surrounding vegetation (“liberated plants”). All vegetation (shrubs, grasses, lianas etc.) was removed in a radius of 0.5 m around the stem of the selected individuals, from soil level until the top of the surrounding vegetation, so that their crowns did not interact with the surrounding vegetation. Regrowing vegetation was removed monthly. The vegetation around the other half of the individuals was left intact (“control plants”). After 174 days biomass allocation of all individuals was determined non-destructively (they were part of another ongoing study) so that growth of control and liberated trees could be calculated (for methods and calculations on non-destructive measurements see also Van Kuijk et al., 2008).

Biomass allocation

We measured the following parameters on all study trees: height, leaf angles, length, and diameter of stem, branches and petioles and length and width of leaves. To obtain allometric relations between dimensions and biomass of above ground plant parts, 20 individuals per species were harvested in the same height range as the studied individuals. The same dimensions were measured and dry weight of stem, braches, petioles, and leaves was determined. Dry weights and estimates based on dimensions were correlated and the function that best described dry weight (r^2 varied from 0.92 to 0.99; data not shown) (but see Van Kuijk et al., 2008) was used to calculate dry weight of studied trees. Leaf area was measured with a digital photograph (SigmaScan Pro 5.0).

Photosynthetic characteristics

In March 2005, photosynthesis measurements were done using an open gas exchange system (CIRAS 2, PP systems, Hitchin, UK) equipped with a LED light source. Up to 28 leaves of varying age (young, medium and old: related to position on the branch) were selected on different individuals (max. three leaves per individual) that were growing outside the plots. Photosynthetic rates were measured early in the morning when stomata were open. Maximum photosynthetic rates were measured at Photosynthetic Active Radiation (PAR) values of 1200–1500 μmol $m^{-2}s^{-1}$. In order to determine dark respiration and quantum yield we varied light from 80 to 0 μmol $m^{-2}s^{-1}$ PAR in steps of 10–20 μmol $m^{-2}s^{-1}$. The CO_2 concentration in the chamber was maintained at 370 ppm throughout all measurements.

Nitrogen content

Calculation of the distribution of light saturated photosynthetic rates in the tree crown was done as a function of the nitrogen (N) distribution (Hirose and Werger, 1987). N content was only measured in the most illuminated leaves in the top of the crown (N_o) by drying sampled leaves in an oven for 72 h at 70°C. Nitrogen content of the leaves was analyzed with a continuous flow analyzer (SKALAR, Breda, the Netherlands) following the Kjeldahl method. We calculated the N distribution in the crown (N_{area}) using the equation proposed by Anten (1997): $N_{\mathrm{area}} = N_{\mathrm{o}}(I/I_{\mathrm{o}})^{0.4}$ (see Equation S11 in Supplementary Material) with I/I_{o} the relative light intensity. This equation shows that the N distribution scales with the light distribution by a power 0.4. It was shown to give good predictions of N distribution in stands of a wide variety of species (Anten, 1997).

Vegetation data

We established 1 m^2 plots in the study area, each containing an individual tree in the center, in which light and LAI were measured. All measurements were done under a uniform overcast sky. Photosynthetic Photon Flux Density (PPFD, 400–700 nm) was measured in the center of each quadrant of each plot, summing up to four light profiles per study tree. These were averaged per plot. Light was measured at ground level, 0.25, 0.5, 0.75 and 1 m using spherical light quantum sensors and meters (LI-250, LiCor). Field testing revealed that light levels higher up in the canopy could be accurately calculated from these values. Simultaneously light measurements were done above the vegetation canopy. The Leaf Area Index (LAI) was measured four times in each plot at ground level from every corner of the (sub)plot facing the center (LAI-2000 Plant Canopy Analyzer, LiCor, NE, USA). Vertical leaf area distribution was determined by counting the number and recording the height of leaves touched by a telescopic rod when moved up through the vegetation. This was done in the center of each quadrant of a plot.

MODEL CALCULATIONS AND SIMULATIONS

The model developed here (PHOLIAGE-model) calculates daily canopy photosynthesis of individual 3D trees in a 3D vegetation stand. It was designed to simulate how the structure of the surrounding vegetation and the pattern in which it can be removed through liberation interact with the physiological and morphological traits of trees in determining whole-tree photosynthetic carbon gain. A detailed description of the model is provided in the Supplementary Material. Here we only describe the basic structure of the model and the simulations that were run.

We assume a tree with specific crown dimensions placed in a vegetation stand with a specific canopy height (**Figure 1**). The length to width ratio of the ellipsoid crown can be varied such that vertically elongated, spherical, and flatter broader crowns can be considered. The canopy of the surrounding vegetation can be fully closed and thus encompass and even overtop the tree, or it can be virtually opened up as a circular gap around the tree. Characteristics of the tree and surrounding vegetation can be varied. With a technique called ray-tracing (Pearcy and Yang, 1996; Rohrig et al., 1999; Bartelink, 2000) the amount of light absorbed in each point in the tree's crown is calculated and subsequently the photosynthetic rate for each point is calculated. An integration over all points is made to calculate whole crown light capture and photosynthetic rate.

Dividing instantaneous whole crown light capture (μmol s^{-1}, Equation S12a) and whole crown photosynthetic rates (μmol s^{-1}, Equation S1) by leaf area, gives the mean light capture per unit leaf area (Φ_{area} in μmol $m^{-2}s^{-1}$) and photosynthetic rates per unit leaf area (P_{area} in μmol $m^{-2}s^{-1}$). This was done to correct for differences in plant size, i.e., a large plant may exhibit a greater total photosynthesis but this may not necessarily be the result of its leaves being positioned more favorably relative to the light gradient or because of a more efficient leaf physiology, which would be reflected in the values of Φ_{area} and P_{area} (Anten and Hirose, 2003; Selaya and Anten, 2010).

We calculated whole-canopy light capture and photosynthetic rates of each individual control tree and of liberated trees immediately after removal of the vegetation in the 1.5 y/o stand. Next,

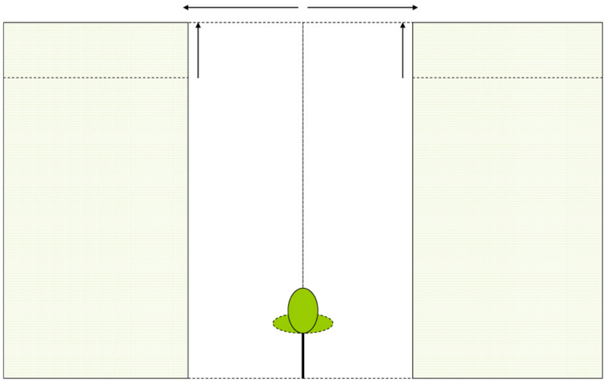

FIGURE 1 | Schematic presentation of a tree and its surrounding vegetation in the PHOLIAGE-model. Characteristics of both tree and vegetation can be varied.

we performed four model simulations. First, we simulated the effect of an increasing gap radius in which vegetation was virtually removed (**Figure 2A**). Second, we simulated the effect of a vegetation removal event (gap radius = 0.5 m) in a vegetation stand with an increasing LAI (**Figure 2B**). Third, the effect of surrounding vegetation height was simulated in a vegetation removal event (gap radius = 0.5 m; leaf area density of the vegetation was kept constant while height varied) (**Figure 2C**). Fourth, we simulated vegetation removal events with variable gap radii in three successional forest stands (i.e., stands with different LAI and height, see **Table 1**).

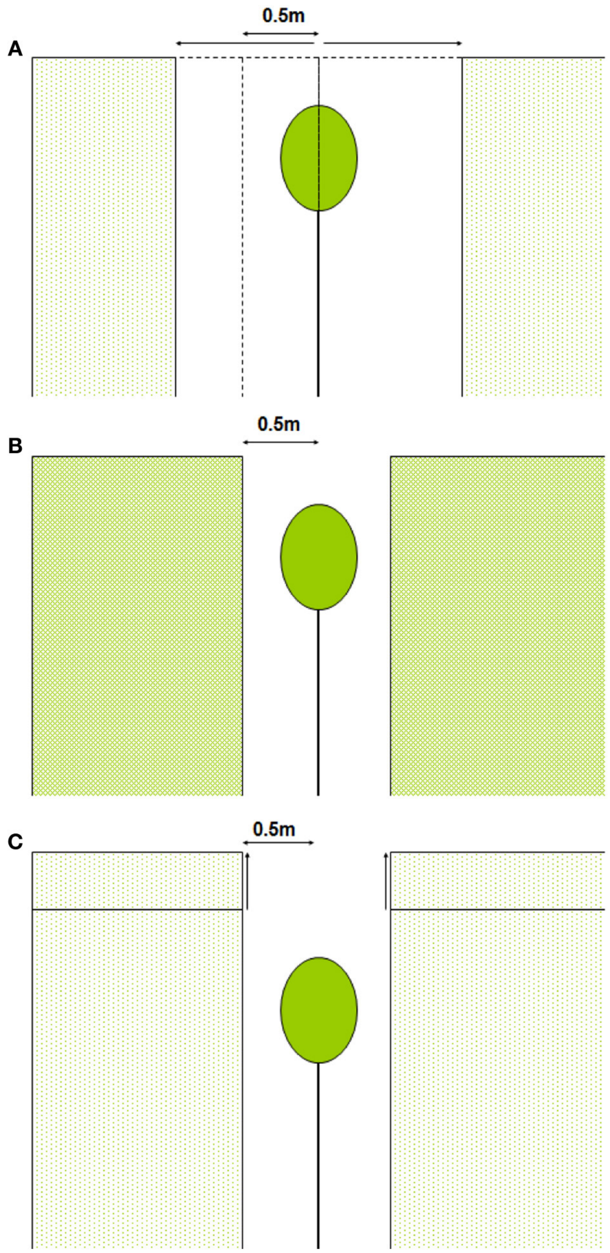

FIGURE 2 | (A–C) Schematic presentation of model simulations. In **(A)** vegetation is removed around the individual within a variable radius, in **(B)** the LAI of the surrounding vegetation is increased and in **(C)** the vegetation height is increased (leaf area density remains constant).

In the first, second and third simulation we used average values for tree height, crown dimensions, leaf area, leaf angle distribution and leaf nitrogen content per species based on all existing individuals of a species in the 1.5 y/o stand. We also assumed average characteristics (LAI, canopy height and leaf angle distribution) of the surrounding vegetation as measured in the study. This was done to analyze the effect of an increasing gap radius, vegetation LAI and vegetation height on species with different dimensions, leaf area and leaf nitrogen content. In the fourth simulation we performed virtual vegetation removal experiments with variable gap radii for individual trees within their surrounding vegetation based on actual field data as measured in a previous study (Van Kuijk et al., 2008). To estimate the extent to which the effect of liberation would depend on the successional age of the vegetation, we simulated tree liberation for three successional stands (0.5, 1, and 1.5 years after field abandonment) whereby the characteristics of these stands and target plants therein were taken from Van Kuijk et al. (2008).

STATISTICAL ANALYSIS

Plots were created after the slash and burn treatment with the sole purpose to be able to trace back the saplings in following measuring periods. However, in this set-up plot-effects might occur. Therefore, analyses were done with Linear Mixed Effects Model (in results section abbreviated as MM).

The effect of species on the average values of light capture, photosynthetic rates and growth were determined by identifying these variables as dependent and species as independent factor. When designing the statistical model, species was the sole fixed effect, including intercept. For random effect an intercept was included for the subject plot number, but no model for random effects was designed. No *post-hoc* tests were available but most between-species differences could be deduced from the parameter estimates.

The relation between observed growth (biomass in g day^{-1}) and calculated photosynthetic rate was analyzed with linear regression and the difference in slopes was analyzed with ANCOVA.

RESULTS

VEGETATION REMOVAL IN THE 1.5 y/o STAND

The average height of the vegetation in the 1.5 y/o stand was 1.4 m and the LAI was on average 3.73 (**Table 1**). Species differed in height but were on average lower than the surrounding vegetation

Table 1 | Characteristics of successional vegetation stands in a Vietnamese forest (average values ± *SE*).

	Stand I	Stand II	Stand III
Age (year)	~0.5	~1	~1.5
Mean LAI (m^{2}m^{-2})	3.03 ± 1.74	5.46 ± 0.91	3.73 ± 0.75
Mean height (m)	0.61 ± 0.18	0.99 ± 0.31	1.40 ± 0.41

Data are taken from Van Kuijk et al. (2008).

(**Table 2**). Average growth characteristics differed between liberated and control trees after liberation (**Table 3**) with the liberated trees growing less in height, but more in biomass.

Model calculations showed that in all species liberated individuals on average tended to have higher absolute whole-canopy light capture rates (μmol s^{-1}) (the horizontal light intensity on top of the canopy was set to 1000 μmol $m^{-2}s^{-1}$) and photosynthetic rates (μmol s^{-1}) immediately after removal of vegetation than the control individuals (**Figures 3A,B**) but none of these differences were significant (MM, $p > 0.05$). Liberated plants had significant higher light capture per unit of leaf area (Φ_{area}) and photosynthetic rate per unit of leaf area (P_{area}) (**Figures 3C,D**) (MM, $p < 0.05$) than control plants, except for P_{area} of *Mallotus microcarpus* (MM, $p = 0.133$).

Relative growth rates in terms of biomass (RGR) and height were measured in the period following the removal of the surrounding vegetation (**Figure 4**). Vegetation removal tended to result in a higher RGR (**Figure 4A**), but this was only significant for *Mallotus paniculatus* (MM, $p = 0.043$). Height growth tended to be lower for liberated plants (**Figure 4B**), but this was only significant for *Macaranga denticulata* (MM, $p = 0.036$).

Model calculations of instantaneous absolute photosynthetic rates immediately after removal of vegetation, were significantly correlated to above ground mass growth (g day^{-1}) for all species in both the control and liberation treatment in the period following removal (**Figure 5**). The slopes of these relationships did not differ significantly between the control and liberation treatment (MM, $p > 0.05$), indicating that the model was well able to predict the effect of liberation on growth. Slopes were higher for *Mallotus paniculatus* than for the other species (MM, $p = 0.011$).

MODEL SIMULATION: VARIATION IN GAP RADIUS

Values of absolute light capture and photosynthetic rates increased with increasing gap radius and reached near-maximum values at a gap radius of approximately 1 m (**Figures 6A,B**). *Mallotus microcarpus* captured the largest amount of light and had the highest photosynthetic rate while *Mallotus paniculatus* captured the least light and had the lowest photosynthetic rate. This corresponded with species height and leaf area (**Table 2**). *Mallotus microcarpus* was the tallest species, the top of its crown reached on average 90% of the surrounding vegetation height, and it had the most leaf area. *Mallotus paniculatus* was the shortest species with a mean height of 67% of the surrounding vegetation height, and it had the smallest leaf area. *Macaranga denticulata* showed intermediate values for light capture, photosynthetic rates, height, and leaf area.

Mallotus microcarpus reached maximum values of light capture (as a percentage of light capture at a gap radius of 2.5 m, which showed maximum values of light capture for all species) before the other species did (**Figure 6C**). This was related to the crown shape of the species and species height. *Mallotus microcarpus* had the smallest crown length compared to its crown width (**Table 2**) and it was positioned relatively high in the canopy. Thus, with increasing gap radius, the bottom part of its crown will be capturing maximum light levels sooner than a crown that is placed lower in the vegetation and has a longer crown length compared to its width like that of *Mallotus paniculatus*. *Macaranga*

Table 2 | Average characteristics of tree species in a 1.5 y/o stand (average values ± *SE*).

Plant characteristics	Height	Biomass	Leaf area	Leaf angle fractions			Crown vol.	Crown l:w	Lad	N_o	P_{max}–N_{area} relation	R_d	Quantum yield
Species	**(m)**	**(g)**	**(m^2)**	**15**	**45**	**75**	**(m^3)**		**(m^2 m^{-3})**	**(mmol m^{-2})**	**Slope**	**(mmol $m^{-2}s^{-1}$)**	**(mmol C $PPFD^{-1}$)**
Mallotus microcarpus	1.26 ± 0.07	52.70 ± 8.48	0.29 ± 0.02	0.23	0.48	0.29	0.04 ± 0.00	1.18 ± 0.03	6.72 ± 0.27	94.28 ± 1.25	0.17 ± 0.05	1.04 ± 0.02	0.03 ± 0.00
Mallotus paniculatus	0.94 ± 0.01	12.22 ± 0.56	0.06 ± 0.00	0.13	0.41	0.46	0.01 ± 0.00	2.00 ± 0.03	4.94 ± 0.15	79.33 ± 0.84	0.10 ± 0.05	0.24 ± 0.01	0.03 ± 0.00
Macaranga denticulata	1.07 ± 0.03	27.22 ± 1.17	0.11 ± 0.00	0.37	0.40	0.24	0.03 ± 0.00	1.42 ± 0.02	4.35 ± 0.15	82.52 ± 0.72	0.18 ± 0.06	0.53 ± 0.01	0.04 ± 0.00

For the data on biomass allocation n = 20, 31, and 31 individuals for M. microcarpus, M. paniculatus and M. denticulata respectively.

For the photosynthesis data n = 15–23, 15–28, and 16–17 leaves for M. microcarpus, M. paniculatus and M. denticulata respectively.

Values are calculated based on individuals in the 1.5 y/o stand (data were also taken from Van Kuijk et al. (2008). Leaf angle fractions are divided into three classes indicated by 15, 45, and 75 degrees, crown l:w is the ratio between the length and the width of the crown, lad is leaf area density, No is maximum nitrogen content of the leaves, Rd is dark respiration.

Table 3 | Average growth characteristics of liberated and control trees 174 days after liberation (average values ± *SE*).

Species	Average height liberated trees	Average height control trees	Height growth liberated trees	Height growth control trees	Average biomass liberated trees	Average biomass control trees	Biomass growth liberated trees	Biomass growth control trees
	(cm)	(cm)	(cm/day)	(cm/day)	(g)	(g)	(g/day)	(g/day)
Mallotus microcarpus	180.00 ± 9.51	184.82 ± 9.27	0.31 ± 0.01	0.41 ± 0.02	121.41 ± 12.00	93.56 ± 13.79	0.35 ± 0.03	0.28 ± 0.04
Mallotus paniculatus	121.00 ± 1.58	139.13 ± 2.07	0.19 ± 0.01	0.28 ± 0.01	56.26 ± 4.46	41.12 ± 2.26	0.25 ± 0.02	0.17 ± 0.01
Macaranga denticulata	133.14 ± 3.13	153.65 ± 2.72	0.16 ± 0.01	0.26 ± 0.00	68.57 ± 3.79	54.97 ± 2.87	0.25 ± 0.02	0.16 ± 0.01

For the liberated trees n = 9, 15, and 15 for M. microcarpus, M. paniculatus and M. denticulata respectively.
For the control trees n = 11, 16, and 16 for M. microcarpus, M. paniculatus and M. denticulata respectively.

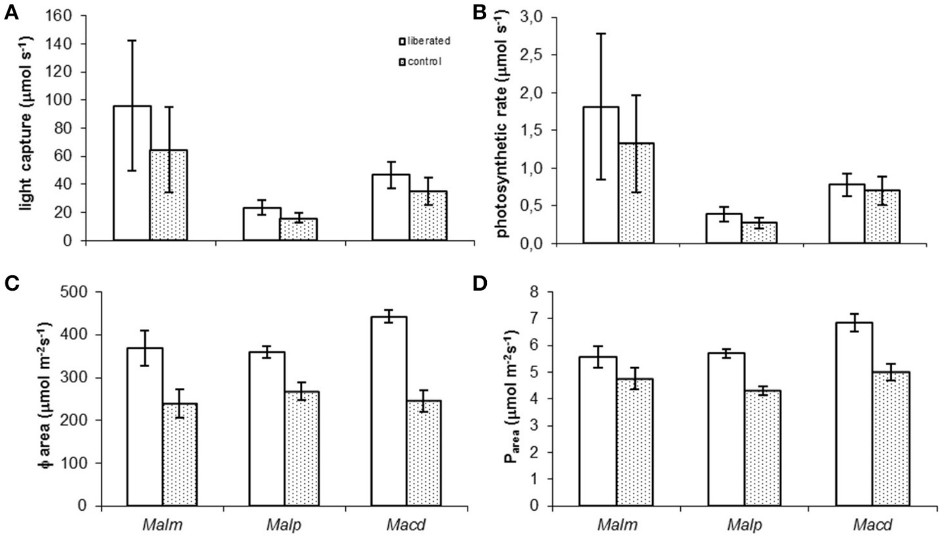

FIGURE 3 | (A–D) Mean values of whole canopy light capture and photosynthetic rate of control and liberated plants immediately after vegetation removal (gap radius = 0.5 m) in a 1.5 y/o secondary forest stand in Vietnam (the horizontal light intensity on top of the canopy was set to 1000 μmol $m^{-2}s^{-1}$). Note that values in **(A,B)** are smaller than the rate per m^2 **(C,D)** as the leaf area of the trees is on average less than 1 m^2. Abbreviations: *Malm, Mallotus microcarpus; Malp, Mallotus paniculatus; Macd, Macaranga denticulata*. Bars denote standard error.

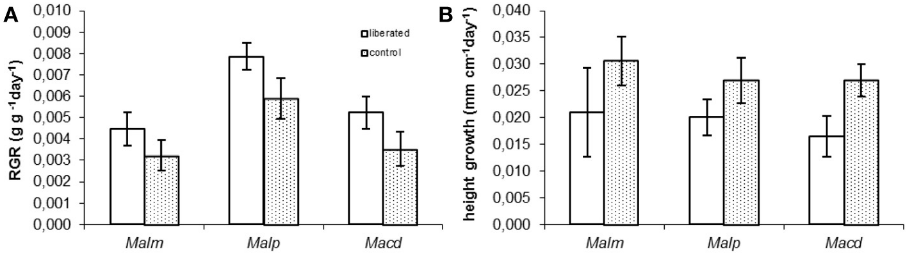

FIGURE 4 | (A,B) Measured above ground Relative Growth Rate (RGR) and relative height growth rate of control and liberated plants in 174 days after vegetation removal (gap radius = 0.5 m). For abbreviations see **Figure 3**. Bars denote standard error.

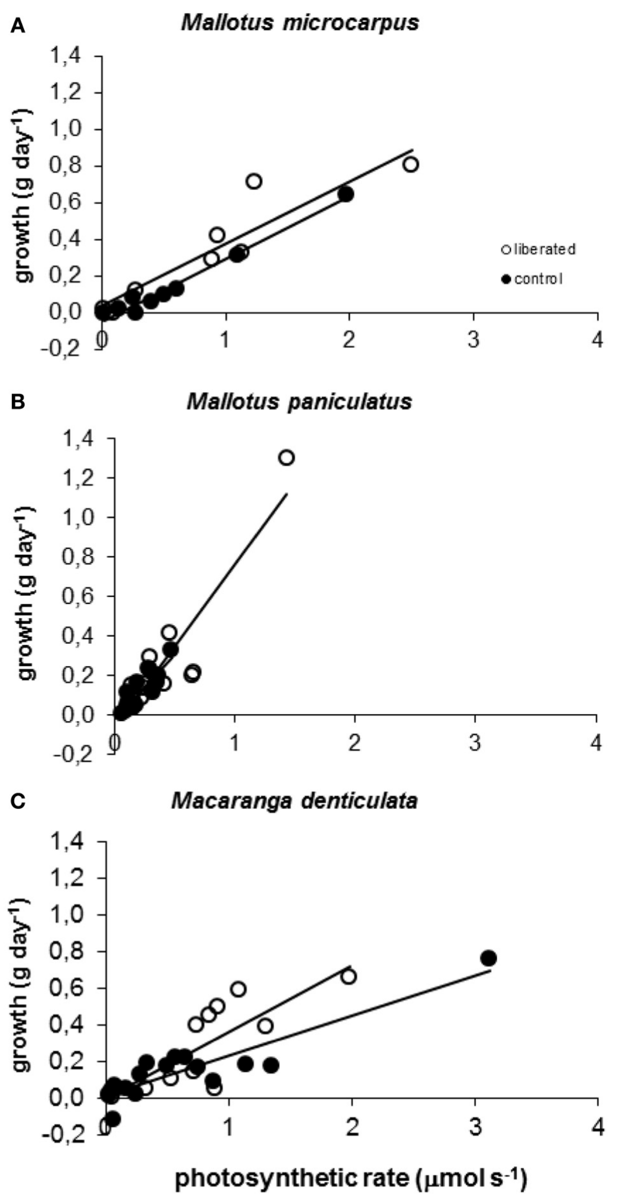

FIGURE 5 | (A–C) Correlation between observed above ground biomass growth in 174 days after vegetation removal and calculated values of whole canopy photosynthetic rates immediately after vegetation removal obtained with the PHOLIAGE model. All correlations are significant (MM, $p < 0.01$) and r^2 values varied from 0.6928 to 0.9744.

denticulata had an intermediate crown size and was positioned at intermediate height in the vegetation and the simulation line in **Figure 6C** fell between that of the other species.

Photosynthetic rate as percentage of the maximum photosynthetic rate was approximately equal for all species at all gap radii (**Figure 6D**). The discrepancy between **Figure 6C** and **Figure 6D** can be explained by the photosynthetic characteristics of the species (**Table 2**). *Mallotus microcarpus* could achieve high levels of maximum photosynthetic rates (high N_o and high slope of the $P_{\max}$ - N_{area} relation) and had a high dark respiration (R_d). *Mallotus paniculatus* on the other hand, was not able to achieve such high levels of maximum photosynthetic rates and its dark respiration was low, which is favorable in low light conditions. *Macaranga denticulata* could also achieve relatively high maximum photosynthetic rates and had intermediate R_d. Thus, the tallest species, *Mallotus microcarpus,* was best able to keep up with the surrounding vegetation height, but was also most light-demanding.

With increasing gap radius, Φ_{area} increased for all species (**Figure 6E**). The smaller increase in *Mallotus microcarpus* can be explained by its relatively short crown and its position relatively high in the canopy. Many leaves already experience high light levels and an increase in gap radius will increase Φ_{area} but not as much as for a tree with a more elongated crown positioned lower in the canopy. The relatively low value of maximum Φ_{area} for *Mallotus microcarpus* is the consequence of its greater leaf area density compared to the other species (**Table 2**). This increases self-shading.

Mallotus paniculatus had the lowest mean photosynthetic rate per unit leaf area (**Figure 6F**). This was related to its relatively low maximum photosynthetic rate. *Macaranga denticulata* showed the highest P_{area}because of its high maximum photosynthetic rates and high Φ_{area}.

MODEL SIMULATION: VARIATION IN LAI AND VEGETATION HEIGHT

When the surrounding vegetation was left intact, photosynthetic rates declined with increasing LAI for all species (**Figure 7A**). The decline was steepest for the species highest in the canopy, *Mallotus microcarpus,* because in absolute terms light availability diminishes more strongly higher in the canopy than lower down, and this species had higher respiration. When vegetation was removed around the individual trees in a radius of 0.5 m, the effect of its LAI on photosynthetic rates was almost completely diminished (**Figure 7B**).

When vegetation height was increased (for average vegetation characteristics see **Table 1**), photosynthetic rates declined in the control and the liberation treatment (**Figure 8**), but the effect for the control plants was stronger than for the liberated plants. *Mallotus microcarpus* reached negative photosynthesis values before the other species did. The sudden decline in photosynthetic rate indicated the moment the height of the surrounding vegetation exceeded that of the target trees.

MODEL SIMULATION: VEGETATION REMOVAL IN SUCCESSIONAL STANDS

In all stands and for all species photosynthetic rate (as a percentage of photosynthetic rate in the control situation: gap radius = 0 m) increased with increasing gap radius (**Figure 9**). In the 1.5 y/o stand the effect of vegetation removal was not as great as in the 1 and 0.5 y/o stands. In the 1.5 y/o stand trees were on average closer to the top of the vegetation than in the younger stands (see Van Kuijk et al., 2008). Average photosynthetic rates in the 1.5 y/o stand were therefore closer to the maximum values than in the same individuals in the younger stands.

In the 1 y/o stand (**Figure 9B**) it seems as if vegetation removal affected photosynthetic rates of *Mallotus microcarpus* less than those of *Mallotus paniculatus* and *Macaranga denticulata*. In the

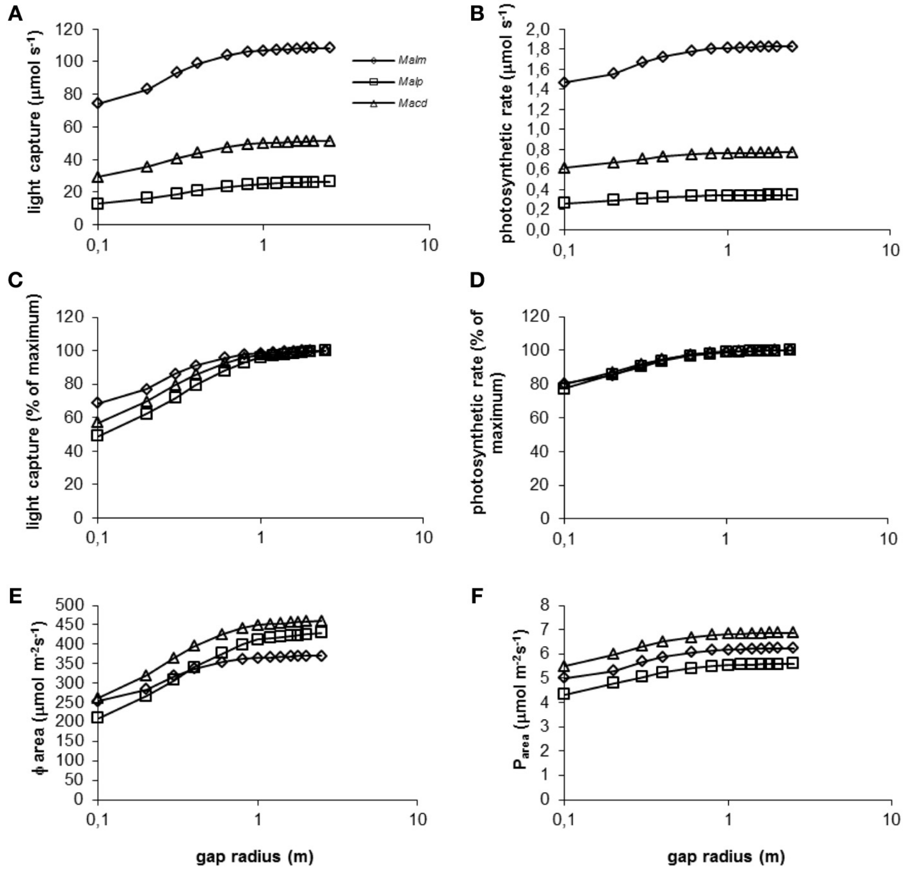

FIGURE 6 | (A–F) Model simulations of the effect of gap radius (log scale) on whole canopy light capture and photosynthetic rate of species in a 1.5 y/o secondary forest stand. Mean parameter values were used for each species and treatment. For abbreviations see **Figure 3**.

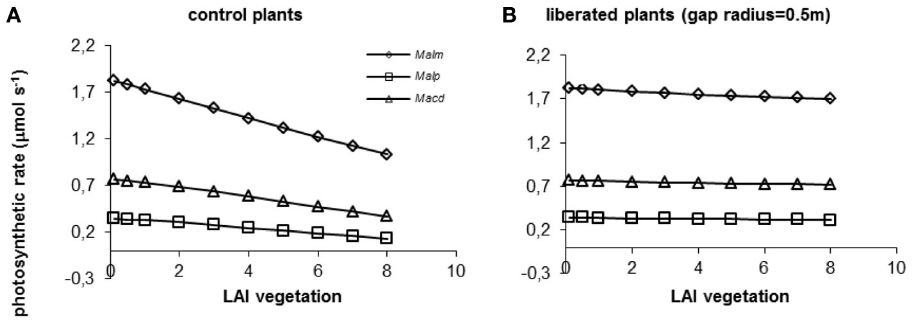

FIGURE 7 | (A,B) Model simulations of the effect of LAI of the surrounding vegetation on whole canopy photosynthetic rates of species in a control situation (surrounding vegetation intact) **(A)** and liberated plants **(B)** in a 1.5 y/o secondary forest stand. Mean parameter values were used for each species and treatment. For abbreviations see **Figure 3**.

0.5 y/o stand (**Figure 9C**) the opposite seemed to occur. In the 0.5 y/o stand all species had approximately the same height respective to the vegetation height (results not shown). *M. microcarpus* had a two-fold higher leaf area and a lower leaf area density than the other species (results not shown), resulting in a higher potential photosynthetic rate. In the 1 y/o stand *M. microcarpus* had the highest height respective to the vegetation height (results not shown), so it was closer to maximum levels of photosynthesis than the other species. Therefore, an increase in gap radius had less effect.

When calculating the increases in photosynthetic rates as percentage of the maximum photosynthetic rates (at a gap radius of

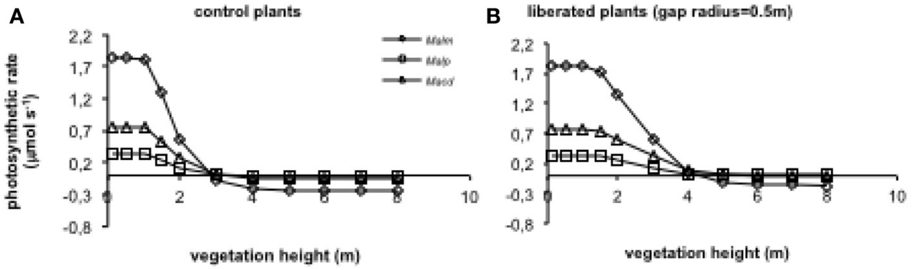

FIGURE 8 | (A,B) Model simulations of the effect of height of the surrounding vegetation on whole canopy photosynthetic rates of species in a control situation (surrounding vegetation intact) **(A)** and liberated plants **(B)** in a 1.5 y/o secondary forest stand (LAI was changed with vegetation height so that leaf area density remained constant). Mean parameter values were used for each species and treatment. For abbreviations see **Figure 3**.

2.5 m), differences between species and stands were small (90–96% with a gap radius of 0.5 m and 98–99% with a gap radius of 1 m, depending on species and stand).

DISCUSSION

SPECIES RESPONSES TO LIBERATION

Our model calculations indicated that removal of surrounding vegetation around target trees in a young secondary forest stand was beneficial in terms of light capture and photosynthetic rate. The field measurements showed that species in this study responded slightly different to the treatment depending on their morphological and physiological characteristics. Model calculations showed that the responses were also dependent on the characteristics of the surrounding vegetation. There were strong correlations between calculated photosynthetic rates and observed growth, indicating that the model presented in this paper was well able to predict the growth of trees in early successional forests. These relationships did not differ significantly for the liberated and control plants. This indicates that the model was capable of predicting the magnitude of liberation effects on tree growth. This model can therefore be utilized to understand how tree species traits and the pattern and timing of vegetation removal together drive the effects of tree liberation. As such it is a first important step toward developing a predictive tool in designing experiments and management scenarios to improve the effectiveness of this intervention measure.

In the 1.5 y/o stand vegetation removal in a gap radius of 0.5 m resulted in higher light capture and higher photosynthetic rate per unit leaf area and a tendency toward higher biomass growth and reduced height growth of the trees as compared to the trees in the control situation. Similar results were obtained by Collet et al. (1998) in an oak plantation. They attributed these differences in growth to the reduction of belowground competition between neighboring plants and the target trees. However, the close correspondence between predicted effects of liberation by the model, which includes only aboveground competition, and the measured effects on growth, suggests that light competition played a more prominent role in our study.

The reduced relative height growth in liberated plants was considerable, 25–40%, and seems counterintuitive since biomass increment was stimulated. Plants typically respond to the proximity of neighbor plants through increased stem elongation, reduction in stem diameter and thus a greater plant height per unit mass (Smith, 1982; Selaya et al., 2007). This response, commonly denoted as the shade avoidance syndrome (Smith, 1982) tends to be stronger in early than in late successional tree species (Gilbert et al., 2001). Liberation, removal of neighbor plants, may thus inhibit stem elongation. This could potentially have a negative impact on the competitive ability of liberated trees if the surrounding vegetation regrows after liberation and gradually fills the gap.

Near-maximum values of light capture and photosynthetic rates were reached at a gap radius of approximately 1 m. Additional cutting in a bigger radius would hardly increase light capture and photosynthetic rates. Korpelainen et al. (1995) found that planted trees showed no additional growth in strips wider than 2 m in a 3 m high tropical forest. The radius (or width in case of a strip) at which near maximum light levels for growth are reached depends on the vegetation height relative to that of the target tree and will thus be different for every forest stand (Korpelainen et al., 1995; Pena-Claros et al., 2002). The optimal radius for a stand could be determined with the model presented here.

The degree to which light capture of plants increased with increasing gap radius differed considerably between species. This could be related to interspecific differences in morphological traits such as leaf area, tree height (i.e., the crown's position in the canopy) and crown dimensions. Crown architecture determines the display of leaves, light interception, and thus carbon acquisition (Bongers and Sterck, 1998). In our study leaf area density appeared to influence light capture per unit leaf area. A high leaf area density results in increased self-shading within the crown and this reduces light capture. Shaded leaves do not necessarily have negative carbon balances but self-shading does lower whole plant carbon gain (Pearcy and Yang, 1996; Sterck et al., 2003). Differences in physiological traits between species explained the response of the photosynthetic rates to increases in gap radius, and to increases in the LAI and height of the surrounding vegetation. *Mallotus paniculatus* plants had lower photosynthetic capacities but also lower rates of dark respiration than the other species, and this favored their net carbon gain at low, but not at high light. Thus, while this species exhibited a greater increase in light capture per unit leaf area with increasing gap radius, it did not similarly show a greater increase in net photosynthesis.

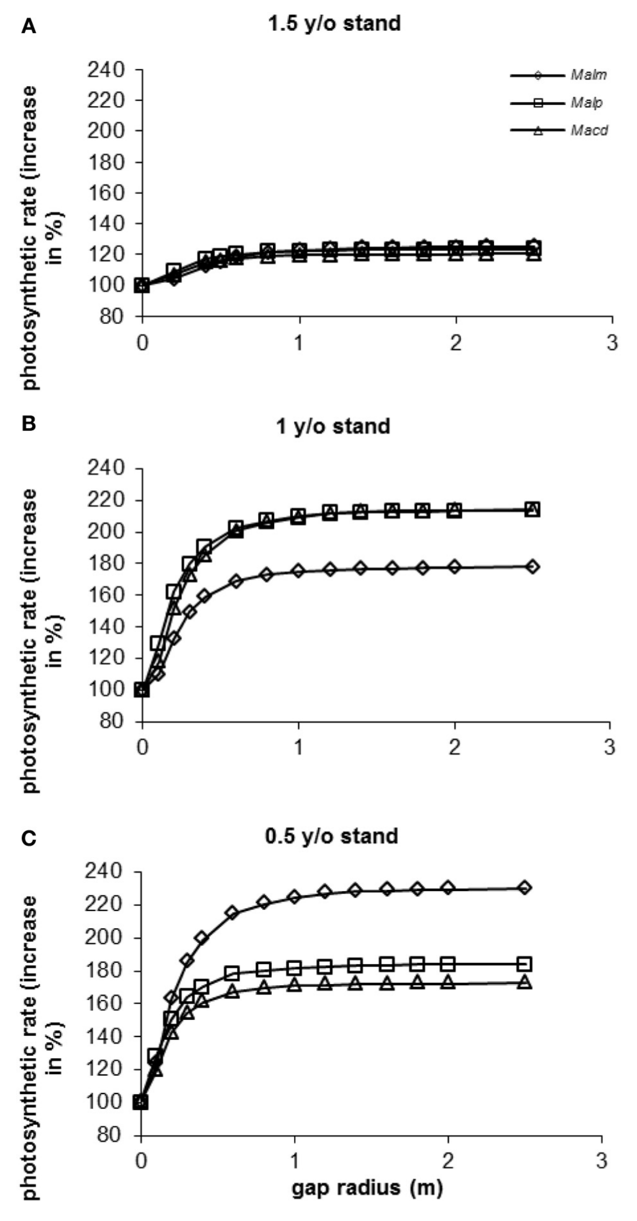

FIGURE 9 | (A–C) Model simulations of the effect of gap radius on whole canopy photosynthetic rates of species in three successional vegetation stands of the same forest. Simulations were done for individual trees [data were taken from Van Kuijk et al., 2008]. For abbreviations see **Figure 3**.

However, a low dark respiration (incorporated in the model) is often correlated to low respiration of other plant parts (not in the model), which might explain the greater carbon use efficiency, i.e., the larger ratio between growth and photosynthesis of *Mallotus paniculatus* as compared to the other two species. We therefore recommend that future model analyses should take respiration of non-photosynthetic tissue into account.

Above mentioned results show that even closely related species, can differ considerably in their optimal light conditions for growth, consistent with previous findings (Ramos and del Amo, 1992; Korpelainen et al., 1995; Montagnini et al., 1997; Dupuy and Chazdon, 2006). In our study it appeared that the species that was best able to keep up with the growing surrounding vegetation was the most light-demanding one. Slower growing species grow in increasingly darker environments since they lack the ability to grow tall rapidly, however, they are also less light-demanding and respond less strongly to increasing light levels. As a result of these two opposing mechanisms the effects of increasing gap radius can be rather similar for species with different light requirements (see **Figure 9A**) growing at different light levels.

ENHANCING FOREST RECOVERY THROUGH LIBERATION

When trees are no longer hampered in growth by the surrounding vegetation, their biomass allocation pattern seems to change. Less biomass is invested in height because the need to grow tall is reduced and instead trees create denser canopies (Collet et al., 1998). This is important in younger successional stands since shrubs, even though they are architecturally constrained to grow tall, can still keep up with tree height and thus overtop trees in such young stands. A denser tree crown will increase the shading out of the shrubs and grasses growing underneath the tree's crown. Once the tree has overtopped and (partly) shaded out the surrounding vegetation, it will be hampered considerably less in growth.

Vegetation removal experiments in forests generally result in increased growth of the liberated trees (Collet et al., 1998; Finegan et al., 1999) but they have not always been successful because in some cases vegetation surrounding trees appeared to facilitate tree recruitment by changing soils conditions (Vieira et al., 1994; Aide et al., 1996; Li et al., 1999). The degree of success of a vegetation removal event depends on many factors such as site characteristics (soil moisture content and nutrient availability) (Putz and Canham, 1992; Li et al., 1999), the light requirement of the target species (Dupuy and Chazdon, 2006; Petritan et al., 2007), the type of plants in the surrounding vegetation (Vieira et al., 1994; Holl, 1998), the number of removal events (De Graaf et al., 1999; Pariona et al., 2003) and the timing of the intervention (Fuhr et al., 2001). Our model yields insights into how the effects of vegetation removal can be mediated by the characteristics of the target species and the vegetation, the radius in which vegetation is removed and the timing of removal events. For this specific forest we showed that the greatest effects of vegetation removal are realized if the vegetation is removed within the first year of stand development. We also demonstrated that increasing the gap radius from 0.5 to 1 m resulted in a relatively small increase in whole-plant photosynthesis. Such an increase in gap radius, however, will require more labor and thus incur more costs. These resources could be spared or could be used to do a subsequent 0.5 m liberation later in time (though we did not test the effects repeated liberation). Even though factors such as soil and climate characteristics are not incorporated in the model, the simulation of vegetation removal in different successional stands is an appropriate practical example that shows the potential use of the model for decisions in the field.

MODEL APPLICATION

The PHOLIAGE model is not the only model that calculates tree photosynthetic rates but is unique in that it is used to approach a practical problem in restoration ecology. In very early stages of tropical forest succession, such as in this study, the PHOLIAGE model approach proved to be effective in predicting growth and we believe it is therefore a first important step toward a predictive tool for estimating effects of tree liberation and similar forest management practices. However, the model still contains a number of simplifications. First interaction for belowground resources is not considered. Liberation does not only entail release from light competition but also partial release from belowground competition. In our case the vegetation surrounding trees was mostly composed of perennial grasses, whereby removal of aboveground parts probably reduces root functioning. Thus, liberated trees not only experienced more light but probably also greater availability of soil resources, though demand for soil resources probably increased. Second, the model did not consider growth dynamics of the study tree (including respiration of non-leaf tissue and shifts in biomass allocation). For example, liberated trees may increase biomass allocation to roots and this could modify their response. These effects should be accounted for in future models to improve generality of application to more situations in tropical regions.

Model parameters are easy to gather relative to some other more detailed growth models (Pearcy and Yang, 1996; Sterck and Schieving, 2007), but we realize it still involves some physiological measurements such as photosynthesis which may not always be available. In those cases and depending on the tree species in question reliable data might be available in databases of species functional traits (Wright et al., 2004). However, care needs to be taken as these traits are plastic and differ genetically within species.

In this study light capture and photosynthesis were calculated instantaneously. If the model were to be applied in non-tropical areas, the larger variation in light climate (during the day and during the year, and related to geographical position) would need to be taken into account. This affects the amount of light a plant receives and consequently plant growth. Also rainfall and soil characteristics may affect growth. If the model is to be used in ecosystems that are located far from the equator (for instance in temperate zones), or in systems that experience strong seasonal effects, or where water or soil factors greatly affect plant growth, it will need to be extended.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fpls.2014.00345/abstract

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Across a macro-ecological gradient forest competition is strongest at the most productive sites

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We tested the hypothesis that the effect of forest basal area on tree growth interacts with macro-ecological gradients of primary productivity, using a large dataset of eucalypt tree growth collected across temperate and sub- tropical mesic Australia. To do this, we derived an index of inter-tree competition based on stand basal area (stand BA) relative to the climatically determined potential basal area. Using linear mixed effects modeling, we found that the main effects of climatic productivity, tree size, and competition explained 26.5% of the deviance in individual tree growth, but adding interactions to the model could explain a further 8.9%. The effect of competition on growth interacts with the gradient of climatic productivity, with negligible effect of competition in low productivity environments, but marked negative effects at the most productive sites. We also found a positive interaction between tree size and stand BA, which was most pronounced in the most productive sites. We interpret these patterns as reflecting intense competition for light amongst maturing trees on more productive sites, and below ground moisture limitation at low productivity sites, which results in open stands with little competition for light. These trends are consistent with the life history and stand development of eucalypt forests: in cool moist environments, light is the most limiting resource, resulting in size-asymmetric competition, while in hot, low rainfall environments are open forests with little competition for light but where the amount of tree regeneration is limited by water availability.

Keywords: climate, diameter increment, tree size, competition, basal area, *Eucalyptus*

INTRODUCTION

Tree growth rates are influenced by many factors including climate and edaphic properties, tree size, and the competitive environment (Gómez-Aparicio et al., 2011; Craine and Dybzinski, 2013). Ranking the relative importance of these factors presents formidable practical challenges given the complexity of the interactions and the spatial and temporal scales involved in working with trees. Macro-ecological studies using extensive networks of permanent forest plots offer the opportunity to investigate trends in growth responses over large spatial and temporal scales, and across a wide range of species and environmental conditions to discern the relative effects of species, tree size, competition, climate, and edaphic factors (Canham et al., 2006; Kunstler et al., 2011; Gómez-Aparicio et al., 2011).

We have assembled a dataset of tree growth measurements from 2409 plots in temperate Australia to undertake macro-ecological research into tree growth. We have found that eucalypt growth is positively correlated with water availability but negatively related to mean annual temperatures in excess of 11°C (Bowman et al., 2014). We have also demonstrated that eucalypt growth is subject to a strong negative interaction between temperature and tree size (Prior and Bowman, 2014). Our data present an opportunity to use statistical modeling to assess the relative influence on eucalypt growth of inter-tree competition, climate, and tree size across a wide productivity gradient.

Competition is the process by which two or more individuals acquire resources from a common, potentially limiting supply (Craine and Dybzinski, 2013). Grime (1977) theorized that the importance of competition in unproductive habitats is small relative to the impact of the abiotic constraints on plant growth. We therefore expected that growth would be most sensitive to competition in the most productive environments, manifest in a negative climatic productivity by competition interaction.

Competition can be broadly separated into above ground competition for light, and below ground competition for water and nutrients. Above ground competition is often considered to be asymmetric, because larger trees are able to capture a disproportionately large share of light through shading of smaller trees (Schwinning and Weiner, 1998; Craine and Dybzinski, 2013). On the other hand, competition for water and nutrients is generally assumed to be more symmetric, with the soil volume depleted of these resources being approximately proportional to plant size (Schwinning and Weiner, 1998; Craine and Dybzinski, 2013). The intensity and degree of size-asymmetry in competition falls on a continuum, and is likely to change along a gradient of site productivity (Schwinning and Weiner, 1998; Van Breugel et al., 2012).

Stand basal area (stand BA), which incorporates the number of trees in a stand and their diameters, is a frequently-used index of inter-tree competition in both local scale and regional studies

(Weiskittel et al., 2011). It often performs similarly to more complex, distance-dependent measures of competition (Nyström and Kexi, 1997; Kiernan et al., 2008; Stage and Ledermann, 2008). However, because local and regional effects of BA can offset each other across macro-ecological gradients, studies of inter-tree competition and tree growth must account for differences in site productivity. For example, in northern Australia, a strong negative effect of stand BA on eucalypt growth was detected in a local-scale study (Prior et al., 2006), but not in a regional study that spanned a 500 mm rainfall gradient (Murphy et al., 2010). In the local-scale study, high stand BA at a local scale was associated with increased competition and therefore reductions in tree growth, but at a regional scale higher BA was associated with improved site quality and thus was correlated with increased tree growth.

Weiskittel et al. (2011) noted that stand BA is not a true measure of competition unless it is combined with some measure of stand development. However, the term "stand development" implies a stand is single-aged, and frequently regenerating, multi-aged stands may also be stocked below their climatic potential as a result of disturbances such as fire, storm damage or disease. So in addition to stand BA, we developed an index of inter-tree competition that accounts for actual stand BA relative to site productivity, irrespective of whether a stand is single-aged or multi-aged. We termed this "relative basal area" (RBA), defined as the ratio of actual stand BA to climatically determined potential stand BA, square-root transformed. We reasoned that below-ground competition should be more closely related to water availability and thus RBA, but that above-ground competition should be more closely related to absolute stand BA than to RBA, because incident light flux is not directly related to climatic productivity (water availability and temperature). In other words, a particular stand BA value may represent similar competition for light in high and low productivity environments, but represent greater competition for soil water (and a higher RBA) in low productivity environments.

Here, we investigate the relative importance of climate, tree size, and competition for growth rates of eucalypts at a continental scale, comparing stand BA and RBA as proxies for competition. We also use interactions between these factors to analyze how the intensity of competition, and the degree of size-asymmetry in competition, varied across the climatic productivity gradient, reasoning that the larger the asymmetry, the larger the positive interaction between size and stand BA, We discuss these patterns of competition across productivity gradients in relation to the ecology of eucalypts.

MATERIALS AND METHODS

TREE SIZE AND GROWTH MEASUREMENTS

Permanent growth plots have been established to monitor tree growth in temperate forests by Australian state government forestry organizations since the 1930s (Bowman et al., 2014). Our study focused on permanent plots located in temperate mesic eucalypt forests, defined as forests outside the tropics that receive >500 mm mean annual precipitation (**Figure 1**). Our plots spanned a gradient in mean annual precipitation of 558–2105 mm and mean annual temperature of 6.4–22.4°C. The most productive forests in the study region are located in cool, moist areas of south-eastern Australia, and are among those with the highest biomass on earth (Keith et al., 2009) (**Figure 1**).

The plots are naturally regenerating, often with a pulse of recruitment following disturbance such as wildfire. Some forests have been thinned or logged at various times, which provides a spectrum of sizes, ages, and intensity of competition across the continent. Plots were re-measured after thinning, so the reduction in stand BA is incorporated in our dataset, and our analysis captures its effects on growth. These forests are generally multi-aged, but the age of most trees is not known. Approximately one-third of plots contained only one species of eucalypt >10 cm diameter.

The tree growth data consisted of repeated measurements of the diameter at breast height (DBH) of individually identified trees within marked plots of known area and location with measurement intervals averaging 4.0 years (range 1–44 years) (Bowman et al., 2014). In most cases, all trees >10 cm DBH within a plot were measured, but in some plots only large trees (e.g., >50 cm DBH) were measured over the entire plot, and smaller trees measured in sub-plots of known area. Diameter increments were annualized. For our analyses, we used only eucalypt growth data complying with the following conditions: measurement interval ≥1 year; plot size $\geq 100\ \mathrm{m}^2$; stand BA 10–100 $\mathrm{m}^2\ \mathrm{ha}^{-1}$; eucalypts with DBH from 10–150 cm, and diameter increments from −0.5–2.5 cm year^{-1}. This filtering was done to avoid gross measurement/recording error, very high stand BAs arising from a very large tree on a small plot, and plots that had very recently been clear-felled. After filtering, the dataset comprised records from 2409 plots, and 499,161 tree–intervals and >100 species or subspecies.

In most cases, the spatial configuration of trees within plots was not specified, so it was not possible to compare the effect of competition from larger neighbors with that of competition from all neighbors (e.g., Coomes and Allen, 2007). However, the degree of the size-asymmetry of competition can be inferred from the interactive effect of tree size and competition on growth rates, given that in any stand, large trees will have fewer larger neighbors than the small trees have.

EXPLANATORY VARIABLES

We used linear mixed effects modeling to describe the growth effects of climate, tree size and inter-tree competition, as well as their interactions. In order to do this, for each of the three factors we derived the following single measures to which growth displayed an approximately linear response (**Figure 2**).

Climatic productivity

Climatic data were obtained from the WORLDCLIM dataset (Hijmans et al., 2005) for the BIOCLIM variables mean annual precipitation (P) and mean annual temperature (T). Pan evaporation (E) was derived from ANUCLIM 6.1 (Australian National University, Canberra).

The response of eucalypt growth to climate variables is complex and non-linear (Bowman et al., 2014). We therefore derived an index of climatic productivity to which eucalypt growth showed an approximately linear response. This was based on a generalized additive model of eucalypt diameter growth (in cm

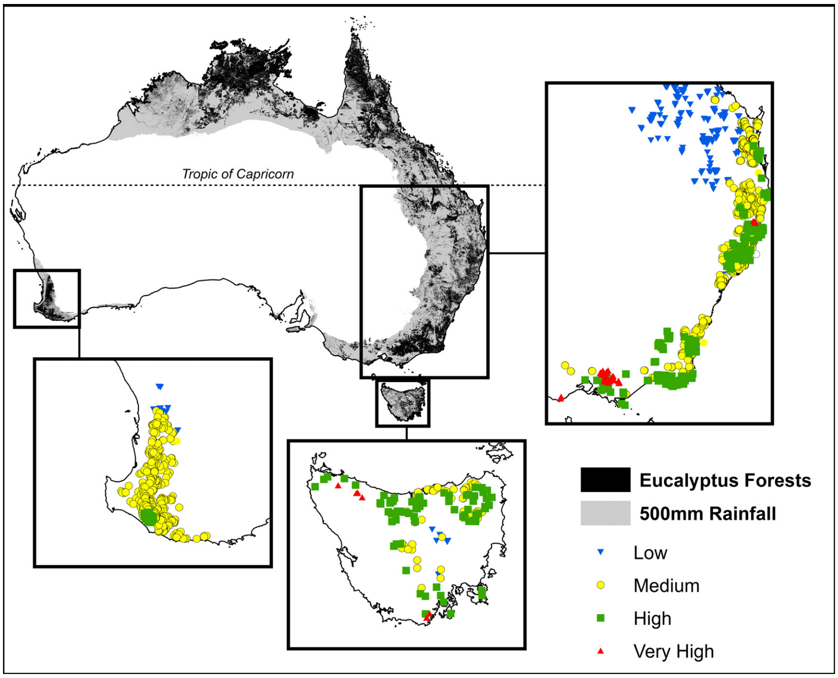

FIGURE 1 | Location of plots showing climatic productivity categories, derived from maximum temperature of the warmest month and the ratio of precipitation to evaporation. These plots were all located outside of the tropics in areas receiving >500 mm mean annual precipitation (shown in gray). Climatic and growth characteristics of these plots are summarized in **Table 1**.

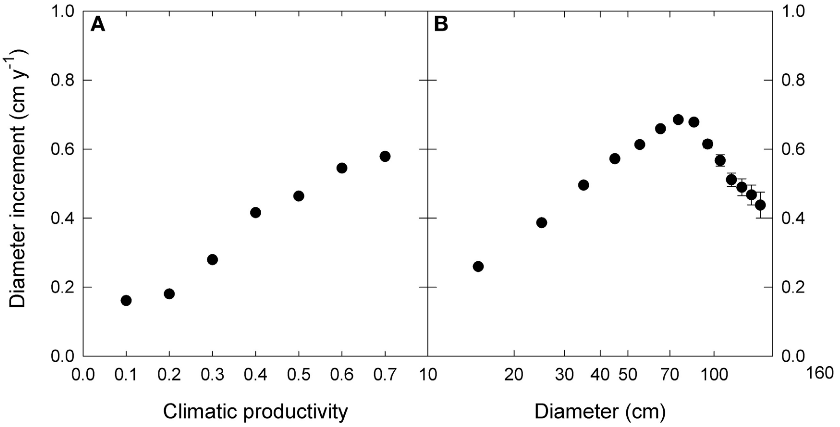

FIGURE 2 | The relationship between diameter increment and (A) climatic productivity index and (B) initial diameter. Note the logarithmic scale for diameter. For presentation, data were grouped into 0.1- climatic productivity index classes and 10 cm—diameter classes. Standard errors are shown where larger than the symbols.

year^{-1}) in relation to T (mean annual temperature) and P:E (the ratio Precipitation: Evaporation, an index of water availability) (Bowman et al., 2014). (We used the model containing T rather than maximum temperature of the warmest month because this better describes the growth response at the cool end of the data range.) This climatic productivity index model was based on plot growth means, and explained 24% of the deviance in growth rates (**Figure 3**). The R package mgcv (v.1.6-2) was used for the generalized additive modeling. Low, medium, high and very high climatic productivity plots were considered to be those with a climatic productivity index of ≤0.2, 0.2–0.4, 0.4–0.6, and >0.6, respectively. The geographic patterning of climatic productivity is shown in **Figure 1**, and temperature, rainfall, water availability and stand characteristics of the climatic productivity classes are summarized in **Table 1**.

Tree size

Initial DBH was used as the measure of tree size. It was log-transformed to normalize the data. We also calculated the coefficient of variation (CV) of the log-transformed DBH of

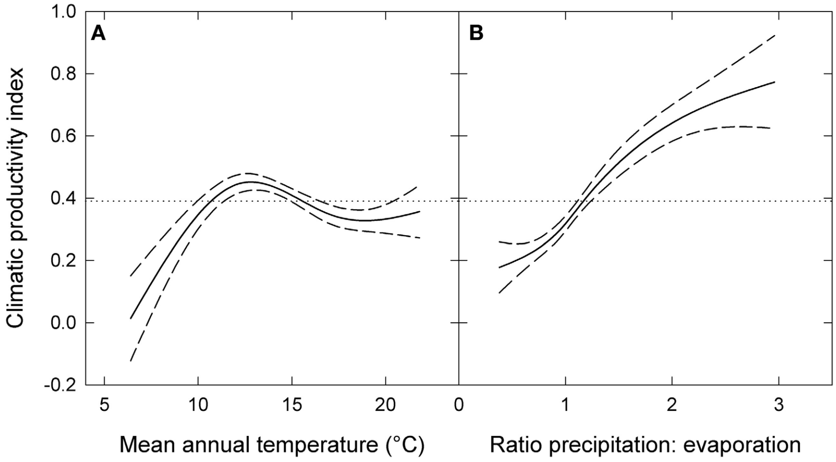

FIGURE 3 | Climatic productivity index as a function of (A) mean annual temperature and (B) the ratio of precipitation to evaporation. The index was based on a generalized additive model describing eucalypt growth (in cm year^{-1}) in relation to these variables. Dashed lines indicate 95% confidence intervals and the dotted lines show mean growth rate for the entire dataset. Responses to each variable were calculated by holding the other variable constant at its mean value.

Table 1 | Summary of climatic conditions, stand basal area, and tree size in the four Climatic Productivity Categories, defined according to growth rate predicted from mean annual temperature and the ratio of precipitation to evaporation.

	Units	Climatic productivity category							
		Low		Medium		High		Very high	
		Mean	***SE***	**Mean**	***SE***	**Mean**	***SE***	**Mean**	***SE***
Climatic productivity index (predicted growth)	Cm year^{-1}	0.15	0.0001	0.31	0.0001	0.49	0.0001	0.66	0.0001
Diameter increment	Cm year^{-1}	0.16	0.001	0.28	0.001	0.48	0.001	0.56	0.001
Mean annual temperature	°C	19.4	0.009	16.6	0.007	12.9	0.005	11.2	0.002
Mean maximum temperature of the warmest month	°C	31.7	0.009	27.4	0.006	24.7	0.004	24.2	0.003
Mean annual precipitation	mm	750	0.7	1107	0.5	1168	0.5	1493	0.5
Ratio precipitation: evaporation	Dimensionless ratio	0.54	0.001	1.00	0.0004	1.38	0.001	2.03	0.001
Initial stem diameter	cm	27.6	0.10	31.6	0.05	29.3	0.04	37.7	0.05
Stand basal area	m^2 ha^{-1}	15.3	0.04	27.3	0.03	39.6	0.04	47.0	0.05
Stand basal area—90th percentile	m^2 ha^{-1}	26.8	0.03	40.6	0.03	57.3	0.02	69.3	0.02
Relative basal area	Dimensionless ratio	0.75	0.0007	0.81	0.0004	0.82	0.0004	0.81	0.004

all trees in a plot the first time it was measured, to examine the relationship between variability in tree size and climatic productivity.

Inter-tree competition

The stand BA of each plot (an indicator of inter-tree competition) was calculated by summing the cross-sectional area of each tree stem, then dividing by the ground area. Where sub-plots were used, stand BA was calculated for each size class individually, then summed to give total stand BA for the plot (trees of all species were included in the stand BA calculations).

Stand BA was correlated with T and P:E (**Table 2** and Bowman et al., 2014). To express stand BA relative to the climatically-determined potential for each plot, we first calculated the 90th percentile of stand BA (BA90) as a function of T and P:E, using the R quantreg package:

$$\mathrm{BA.90} = 90.6 - 3.55 * \mathrm{T} + 9.11 * \mathrm{P} : \mathrm{E}$$

RBA was then calculated as the square root of (stand BA/BA.90). The square root transformation was used to normalize the data. We note that stand BA is also influenced by soil fertility and physical characteristics, and that by definition, 10% of plots will exceed the 90th percentile. Thus the RBA of some plots was > 1.0.

DATA ANALYSES

The magnitude and importance of the effects of climate, tree size and competition, and their various interactions, was investigated using linear mixed effects modeling and model selection based on a robust form of Akaike's information criterion (AICc), a

Table 2 | Correlation matrix for growth, tree size, climatic, and competition variables.

	Diameter increment	l.DBH	CV-l.DBH	Stand BA	BA.90	RBA	*P*	*T*	Max warm	Min cold	*E*	*P:E*	Climatic productivity
Diam incr	1.00	0.31	−0.45	−0.13	0.26	−0.33	0.16	−0.24	−0.22	−0.19	−0.27	0.25	0.28
l.DBH	0.31	1.00	0.03	0.11	0.16	0.03	0.23	−0.13	−0.09	−0.11	−0.10	0.21	0.17
CV−l.DBH	−0.45	0.03	1.00	0.03	−0.36	0.24	−0.17	0.31	0.27	0.23	0.37	−0.39	−0.45
Stand BA	−0.13	0.11	0.03	1.00	0.55	0.79	0.26	−0.55	−0.49	−0.45	−0.56	0.45	0.53
BA.90	0.26	0.16	−0.36	0.55	1.00	−0.03	0.49	−0.98	−0.85	−0.84	−0.94	0.86	0.88
RBA	−0.33	0.03	0.24	0.79	−0.03	1.00	0.00	0.02	−0.02	0.06	−0.02	−0.03	0.03
P	0.16	0.23	−0.17	0.26	0.49	0.00	1.00	−0.33	−0.37	−0.19	−0.35	0.82	0.70
T	−0.24	−0.13	0.31	−0.55	−0.98	0.02	−0.33	1.00	0.85	0.87	0.94	−0.74	−0.80
Max warm	−0.22	−0.09	0.27	−0.49	−0.85	−0.02	−0.37	0.85	1.00	0.68	0.87	−0.67	−0.73
Min cold	−0.19	−0.11	0.23	−0.45	−0.84	0.06	−0.19	0.87	0.68	1.00	0.78	−0.60	−0.62
E	−0.27	−0.10	0.37	−0.56	−0.94	−0.02	−0.35	0.94	0.87	0.78	1.00	−0.77	−0.85
P:E	0.25	0.21	−0.39	0.45	0.86	−0.03	0.82	−0.74	−0.67	−0.60	−0.77	1.00	0.91
Climatic productivity	0.28	0.17	−0.45	0.53	0.88	0.03	0.70	−0.80	−0.73	−0.62	−0.85	0.91	1.00

Variables shown are Diam.incr., annual diameter increment, l.DBH, log-transformed diameter at breast height, CV-l.DBH, coefficient of variation in l.DBH, stand BA, stand basal area, BA.90, climatically determined 90th percentile basal area, RBA, relative basal area, P, mean annual precipitation, T, mean annual temperature), Max Warm, average daily maximum temperature of the warmest month, Min Cold, average daily minimum temperature of the coldest month, E, evaporation, P:E, the ratio of P to E, and the climatic productivity index. n = 499,161 tree—intervals.

model selection index favoring both model fit and model simplicity (Burnham and Anderson, 2002). To establish the importance of the interactions, together and individually, we compared the model containing the three main effects and all two-factor interactions with models containing the three main effects but only one interaction and the model with three main effects but no interactions. Finally, we added the three-factor interaction (climatic productivity × size × competition) to the model with the three two-factor interactions to assess whether size-asymmetric competition was more important in the most productive environments. Stand BA was used as the proxy for competition in one set of candidate models, with the analysis was repeated using RBA. Plot was a random effect in all the models to account for the spatial autocorrelation of individual tree growth. We present results of the analysis that used data from only those trees <70 cm diameter, for which the growth response is approximately log-linear (Prior and Bowman, 2014). The results were very similar, but less deviance was explained, when the full dataset was used. We also investigated whether diameter increment should be log-transformed in the analyses (with an offset for negative values). However, these linear mixed effects models were problematic, with positive log likelihoods and negative % deviance explained. We found that the direction and importance of the effects were similar to the models using untransformed diameter increment, giving us confidence in our conclusions based on untransformed diameter increment.

The sensitivity of individual species to competition was assessed from the slope of the relationship between diameter increment and stand BA for the 30 species with >1600 observations. We present this analysis for small trees only (<30 cm diameter), because these are most sensitive to competition and are well-represented for all major species in our dataset. Results were similar but noisier when trees of all sizes were used.

The statistical software R was used for all analyses (R Development Core Team, 2013). The R package lme4 was used for the linear mixed effects modeling.

RESULTS

Tree diameter, diameter increment, and stand BA increased, but variability in tree diameter decreased, with increasing climatic productivity (**Table 2**).

RANKING OF CLIMATE, TREE SIZE, AND COMPETITION

Our analysis showed that tree size and competitive effects had a much stronger influence than climate on individual tree growth. Climatic productivity on its own explained only 0.2% of the deviance in the growth of individual eucalypts over the climatic gradient, which spanned more than 1500 mm in mean annual rainfall and 16°C in mean annual temperature. [The correlation of diameter increment with climatic productivity was higher in the raw data ($r^2 = 0.08$; **Table 2**); some of this was apparently subsumed by the random effect, plot]. Local site and individual tree factors appeared to have a stronger influence than climate on tree growth, as RBA alone explained 9% of the deviance and tree diameter explained 12% (**Table 3**). Combining the three factors climatic productivity, tree diameter and RBA improved the explanatory power of the modeling. The additive model containing the three factors climatic productivity, initial diameter and RBA explained 26% of the deviance in the growth data, more than the sum of the deviance explained by the individual factors (∼21%; **Table 3**).

RBA vs. BA AS COMPETITION PROXIES

Stand BA was moderately correlated with climatic variables ($r^2 = 0.07$ to 0.31), especially those relating to temperature and evaporation (**Table 2**; **Figure 4**). By contrast, RBA was essentially

Table 3 | Comparison of linear mixed effects models describing individual tree growth.

Model	*K*	Comp = stand basal area		Comp = RBA	
		Delta AIC_c	Deviance expl. (%)	Delta AIC_c	Deviance expl. (%)
Comp * Climatic productivity * l.DBH	8	0	34.47	0	35.37
Comp * Climatic productivity + Comp * l.DBH + Climatic productivity * l.DBH	7	11	34.47	576	35.23
Comp + Climatic productivity * l.DBH	5	3018	33.73	5562	34
Comp * l.DBH + Climatic productivity	5	15166	30.74	32742	27.33
Comp * Climatic productivity + l.DBH	5	34836	25.91	35055	26.76
Comp + Climatic productivity + l.DBH	4	35033	25.86	36023	26.52
l.DBH	2	91555	11.98	95226	11.98
Comp	2	107787	7.99	107792	8.89
Climatic productivity	2	139696	0.16	143368	0.16
Intercept only	1	140329	NA	144001	NA

Explanatory variables were Comp, competition, climatic productivity index, l.DBH, log-transformed diameter at breast height, and the stated interactions. The proxy used for competition was either stand basal area or RBA, relative basal area. Plot was a random effect in all models. The analyses were performed on only those trees <70 cm diameter, where the growth response is approximately linear and positive. The global model, which included all three two-factor interactions, was clearly the best, given that models with a delta AIC > 10 have essentially no statistical support (Burnham and Anderson, 2002). K is the number of parameters in the model and percent deviance explained is relative to the null (intercept only) model. (n = 475,821).

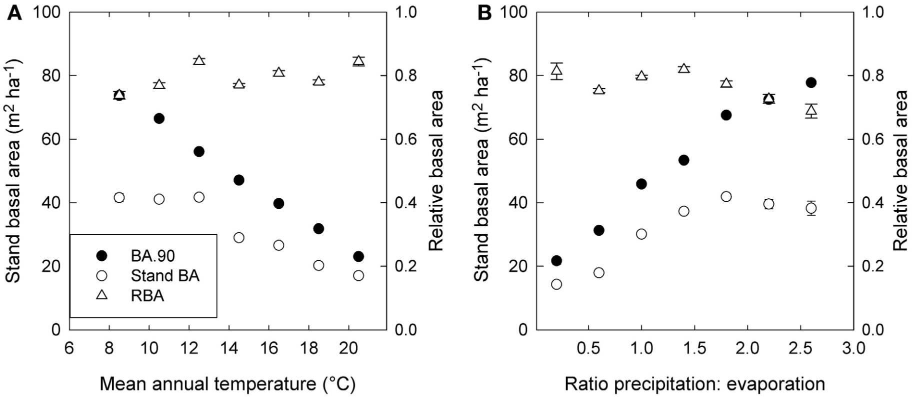

FIGURE 4 | Trends in stand basal area (Stand BA), the 90th percentile of basal area (BA.90) and relative basal area (RBA) in relation to (A) mean annual temperature, and (B) the ratio of precipitation to evaporation (P.E). Standard errors are shown where larger than the symbol. Values are based on plot means, and for presentation are grouped into 2°—temperature classes and 0.4—P.E classes.

uncorrelated with any climatic variables (**Table 2**), supporting its use as a measure of stand development that was independent of climate.

The raw data showed only a slight decline in growth with increasing stand BA, but a much stronger decline with RBA (**Figure 5**). Modeled growth responses were similar for stand BA and RBA (**Figure 6**), but models with RBA generally had better explanatory power, so we focus on describing responses to RBA (**Table 3**).

Although models with RBA were generally superior to those with stand BA, there was an exception regarding the interaction between competition and tree size (an indicator of asymmetric competition). The (stand BA*DBH + climatic productivity) model explained substantially more deviance than the (RBA*DBH + climatic productivity) model (31% *cf* 27%) (**Table 3**).

INTENSITY AND SIZE-ASYMMETRY OF COMPETITION IN RELATION TO CLIMATIC PRODUCTIVITY

Adding the three two-factor interactions boosted the deviance explained to 35% (**Table 3**). There was strong statistical support for all two-factor interactions, given that all models with interactions outranked the model with only the three additive terms (**Table 3**). The most important interaction was the positive climatic productivity by DBH interaction, whereby small trees are relatively insensitive to climate but large trees grow much faster in cool moist climates than in hotter, drier ones (**Figure 7**; **Tables 3**, **4**).

In addition, there was a positive interaction between tree size and RBA, whereby growth of large trees was less affected than that of small ones by a high RBA (**Figure 7**; **Tables 3**, **4**). There was also a negative interaction between climatic productivity and RBA, such that high RBA had the strongest

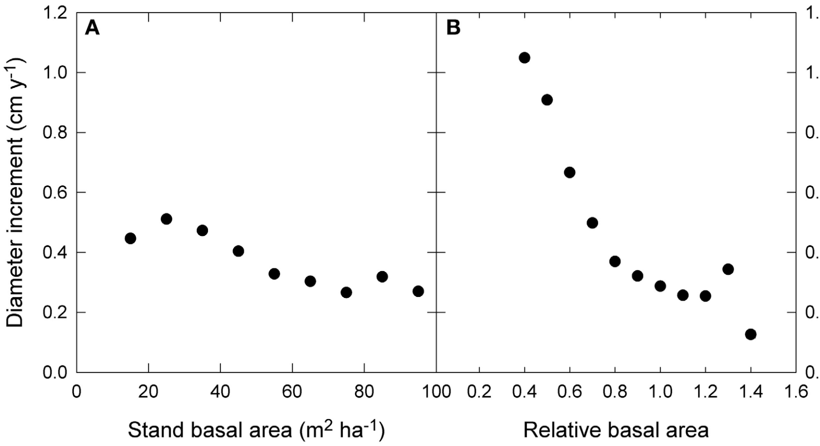

FIGURE 5 | Relationship between diameter growth and (A) stand basal area, and (B) relative basal area. Relative basal area was calculated as square root (stand basal area/the 90th percentile of basal area). The 90th percentile of basal area was estimated by quantile regression of growth as a function of water availability and mean annual temperature. Standard error bars are shown where larger than the symbol.

adverse effect in the most productive environments (**Figure 7**). In other words, competition had the strongest negative effect on growth at the most productive sites: when RBA was high, growth rates of small trees at productive sites were as low as those in low productivity environments. By contrast, tree growth at low productivity sites was uniformly slow, showing little response to either tree diameter or to RBA (**Figure 7**).

As well as the two-factor interactions, there was statistical support for a small, positive three-factor interaction, which explained a further 0.2% of the deviance. Together, these interactions mean that at the most productive sites, large trees were able to grow particularly well, and were better able than small trees to cope with intense competition (**Figure 7**). We were thus able to demonstrate that across a large macro-climatic gradient, size-asymmetric competition was most pronounced at the more productive sites (**Table 4**).

COMPETITIVE RESPONSES IN INDIVIDUAL SPECIES

These responses to tree size and competition (both stand BA and RBA) were apparent in individual species growing in climates with contrasting productivity, as shown by the examples in **Figure 7**. For example, inter-tree competition and response to tree size were particularly pronounced in *Eucalyptus regnans*, which grows mostly in very high productivity environments (**Figure 7**). At the other extreme, *E. fibrosa*, found at sites with low to medium productivity, showed little growth response to either tree size or RBA (**Figure 7**). The stronger growth reductions in the most productive environments were apparent in responses to stand BA as well as to RBA. For instance, across the 30 species with >1600 observations, the slope of the growth-stand BA relationship was most negative in species growing in the most productive environments (**Figure 8**). In other words, for a given increase in stand BA there was a more severe growth reduction in the most productive environments.

DISCUSSION

RELATIVE IMPORTANCE OF CLIMATE, TREE SIZE, AND COMPETITION

We have analyzed a macro-ecological gradient spanning 1547 mm mean annual precipitation and 16°C mean annual temperature. On its own, climate had a surprisingly small effect on tree growth rates, explaining only 0.2% of the deviance in the data. RBA, as a proxy for competition, and tree size both had a much stronger effect, explaining 9 and 12% of the deviance respectively. This is similar to results from a Spanish study, which found that competition for water, light and nutrients from neighboring trees may exert an even stronger influence on tree growth than do tree size and climate (Gómez-Aparicio et al., 2011).

There were strong interactions amongst the three factors, indicating that favorable climates are especially advantageous for the growth of large trees (Prior and Bowman, 2014), but that inter-tree competition, and especially size-asymmetric competition, is most pronounced in these most productive climates, as discussed below.

RBA vs. BA AS A COMPETITION PROXY

Using stand BA as a measure of competition is problematic when site quality varies, as in this study which spanned a very large climatic gradient. This is because high stand BA can be associated with either high productivity (positive relationship with growth) or with strong inter-tree competition (negative relationship with growth), and these two opposing effects can largely offset each other. Creating RBA had the desired effect of virtually removing any correlation with climatic variables, so that it reflected the degree of inter-tree competition relative to environmental productivity and, by extension, to stand development (**Figure 4** and **Table 2**).

ASYMMETRIC AND SYMMETRIC COMPETITION

We found that growth was more closely related to RBA than to stand BA, except with regards to the competition by tree size interaction, which is indicative of asymmetric competition. This

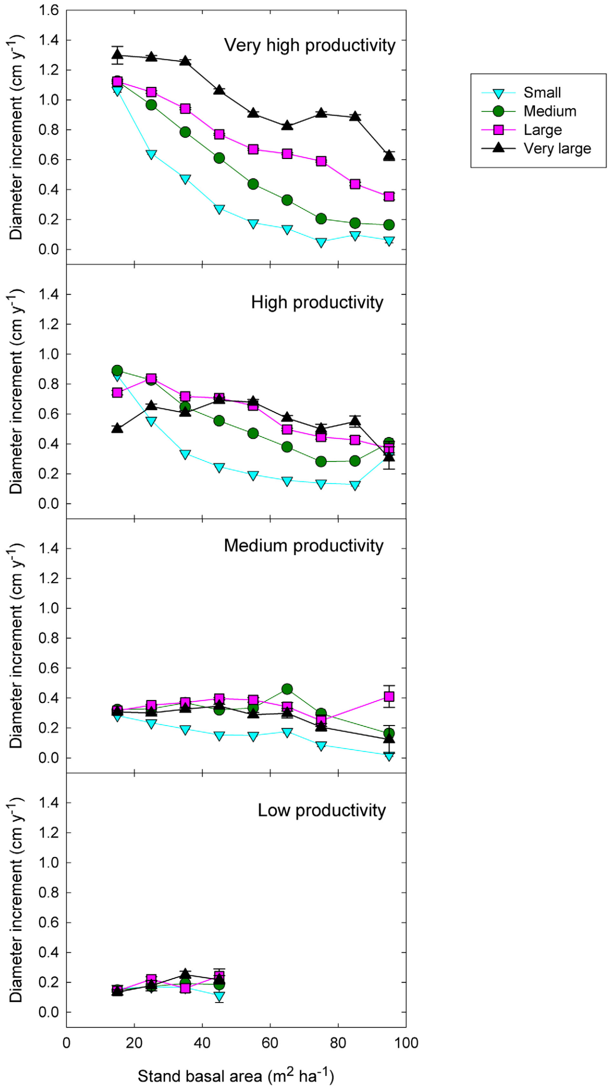

FIGURE 6 | Eucalypt diameter growth in relation to stand basal area, tree size, and climatic productivity category. (This is analogous to the left-hand graphs in **Figure 7**, except that stand BA has been substituted for relative basal area).

interaction was much more pronounced when stand BA was used as the measure of competition than when RBA was used, whether alone or in combination with climate: adding this interaction to the 3-factor additive model explained an additional 4.8% of the deviance in the stand BA models compared with 0.8% for the RBA ones. Our analysis therefore suggests that absolute stand BA is more important than RBA in regards to asymmetric competition, whereby larger plants pre-empt directionally- supplied resources, most notably light (Schwinning and Weiner, 1998). We infer that RBA more closely reflects total competition relative to site quality, while stand BA is a better proxy for competition for light. Over the large climatic productivity gradient in this study, growth appeared more closely related to total competition, presumably because of the strong influence of water availability and temperature.

COMPETITION ACROSS PRODUCTIVITY GRADIENTS

Grime (1977) theorized that competition is most important in productive habitats, and conversely, Bertness and Callaway (1994) postulated that positive biological interactions are more important in physically stressful habitats than in benign ones (the "stress-gradient hypothesis"). However, there have been only a few tests of these ideas for tree growth along large-scale bioclimatic gradients (Craine and Dybzinski, 2013): a New Zealand study showed competitive effects on tree growth declined with increasing altitude (Coomes and Allen, 2007); a French study that demonstrated the relative importance of competition declined with increasing abiotic stress (Kunstler et al., 2011); and a Spanish study contradicting this theory, finding that trees growing in low rainfall areas were more sensitive to competition than those at wetter sites (Gómez-Aparicio et al., 2011). Our results that competition relative to climatic potential, and asymmetric competition in particular (positive size by stand BA by climate interaction), are most pronounced in the most productive climates, provide additional support for Grime's (1977) theory, although the effects are relatively weak. The influence of climatic productivity on competition (manifest in a negative RBA by climate interaction) added only 0.24% to the deviance explained, and the three-factor interaction (suggesting that size asymmetric competition is also most pronounced in the high-productivity environments) boosted deviance explained by a further 0.14%. While mixed species forests may be more productive than pure stands, especially in poor sites (Pretzsch et al., 2013), this is unlikely to be a factor in our results, given the most productive eucalypt forests contained few species of eucalypts or other trees.

Our findings indicate that in the most productive environments, plants compete size asymmetrically early in stand development, as shown by the strong response to tree size when stand BA and RBA were low. The response to tree size was weaker as RBA increased, consistent with decreasing intensity of competition for light, because large trees are less likely to be overshadowed by taller neighbors (Coomes and Allen, 2007). By contrast, there was little effect of tree size in the low and medium productivity environments. Our results are therefore in line with other studies showing stronger competition for light in mesic than in xeric forests, because mesic forests have greater leaf area indices and capture more light (Grime, 1977; Coomes and Grubb, 2000). This has also been predicted to drive strong height growth, in keeping with the occurrence of some of the world's tallest forests in these environments (Coomes and Grubb, 2000; Tng et al., 2012).

EUCALYPT ECOLOGY AND INTER-TREE COMPETITION

We expected that eucalypts, being shade-intolerant (Florence, 1996; Kariuki, 2008; Bond et al., 2012), would be strongly influenced by asymmetric competition (Kunstler et al., 2011).This was indeed the case, as shown by the positive interaction between tree size and competition, which indicates that competition for light suppresses growth of small trees. This is consistent with the ecology of these trees: there is very little eucalypt regeneration in undisturbed wet eucalypt forests, which occur in the most productive environments (Tng et al., 2012; Bowman et al., 2014). On the other hand, size symmetric competition is probably

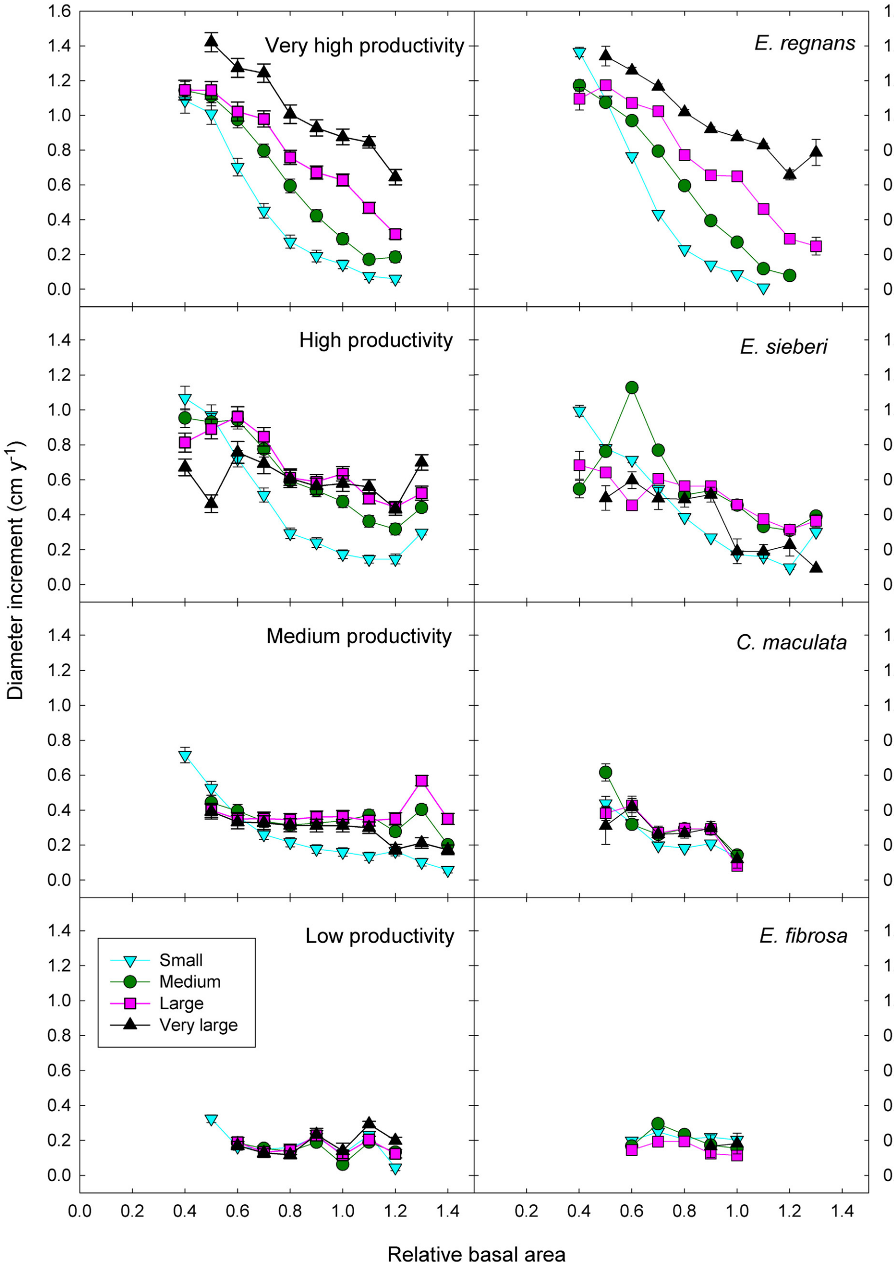

FIGURE 7 | Eucalypt diameter growth in relation to relative basal area, tree size, and climatic productivity category. Data for a representative species growing in the climatic productivity category is presented in the right hand column. There was statistical support for all interactions. Standard errors are shown where larger than the symbol.

most prevalent in productive environments, because stands here contain trees of relatively uniform size.

Eucalypt regeneration is linked to stand to landscape scale disturbances, especially fire. Prevailing disturbance regimes, and thus the competitive relationships of eucalypts, are strongly influenced by climate (Ashton and Turner, 1979; Bowman and Kirkpatrick, 1986; Florence, 1996). For instance, eucalypt forests in the wettest areas typically exhibit massive regeneration following fire, resulting in even-aged cohorts. Initially, extremely dense regeneration competes strongly for light, but the stand rapidly thins as it matures, and competition for light amongst the remaining eucalypts diminishes. Mature wet forest typically consists of emergent eucalypts over an understory of rainforest trees, which are able to regenerate without disturbance (Tng et al., 2012).Our findings also accord with those of Canham et al. (2006), that competition for light has a strong influence on the growth of small trees, while trees of all sizes are affected by competition for nutrients.

Table 4 | Summary of effects on growth of climatic productivity, tree size, and our proxies for competition (stand BA and RBA), and their interactions, from linear mixed effects modeling.

Effect	Direction	Competition proxy (% deviance expl.)		Interpretation
		Stand BA	RBA	
Climatic productivity	+			Best growth in most productive climates
Tree size	+			Large trees grow fastest
Competition	−	8.0	**8.9**	Growth declines with increasing competition, and is more closely linked to competition relative to its climatic potential than to absolute stand BA
Climatic productivity × tree size	+			Large trees grow especially fast in the most productive climates, and are especially affected by unfavorable climates
Climatic productivity × competition	−	0.05	**0.24**	Adverse effect of competition on growth is greatest in the most productive climates. Best correlated with RBA, which reflects competition relative to climatic potential
Tree size × competition	+	**4.9**	0.8	Asymmetric competition—adverse effect of competition on growth is weaker for large trees than for small ones, and more marked for absolute stand BA, which provides a better measure of shading than does RBA
Climatic productivity × tree size × competition	+	<0.01	**0.14**	Asymmetric competition is most pronounced in the most productive climates. Best correlated with RBA, which reflects competition relative to climatic potential

*The direction of effects was the same for both competition proxies, but the magnitude differed, as shown by the % deviance explained (relative to the simpler model without that term). The better proxy for each term is shown in bold. There was statistical support for all effects listed (**Table 3**). Coefficients and standard errors of the key RBA models are listed in **Table 5**.*

Table 5 | Coefficients and associated standard errors for the global model describing eucalypt growth in terms of climatic productivity, tree size, and competition, and their interactions.

Term	Global model		Model with two-factor interactions		Additive model	
	Estimate	*SE*	Estimate	*SE*	Estimate	*SE*
Intercept	0.71	0.06	2.08	0.03	0.18	0.02
Climatic productivity	1.23	0.13	−1.70	0.06	0.82	0.04
l.DBH	−0.22	0.04	−1.17	0.02	0.77	0.003
RBA	0.18	0.08	−1.55	0.03	−1.54	0.01
Climatic productivity × l.DBH	1.00	0.09	3.01	0.02		
Climatic productivity × RBA	−6.10	0.16	−2.40	0.04		
l.DBH × RBA	−0.38	0.05	0.81	0.02		
Climatic productivity × l.DBH × RBA	2.54	0.11				

Adding interaction terms can sometimes reverse the sign of coefficients (For example, DBH is positively correlated with growth, but when the climatic productivity by DBH interaction is added to the model, the sign of the main DBH effect becomes negative), so coefficients from simpler models are presented to indicate the direction of the main effects and two-factor interactions. The coefficients presented here are from models using only trees < 70 cm diameter, for which the growth response is approximately log-linear; those based on the full dataset were similar. Tree diameter was log$_{10}$-transformed.

In drier forests, tree size and stand density appear to be constrained by water availability. In these dry sclerophyll forests, adult eucalypts suppress juvenile eucalypts through competition for water (Rotheram, 1983; Bowman and Kirkpatrick, 1986), and during severe droughts, there is intense competition for water amongst adults, leading to canopy dieback and tree thinning (Fensham et al., 2009; Brouwers et al., 2013; Matusick et al., 2013). This is similar to other work showing that competition in a dry climate leads to widely-spaced dominants (Coomes and Grubb, 2000), and that competition diminishes in importance as abiotic stress increases (Kunstler et al., 2011).

The patterns described above, for eucalypts as a whole, are also evident for individual species (**Figures 7**, **8**). For example, the world's tallest angiosperm, *Eucalyptus regnans*, grows in the highly productive cool moist forests of south-eastern Australia and is one of the most closely studied Australian tree species

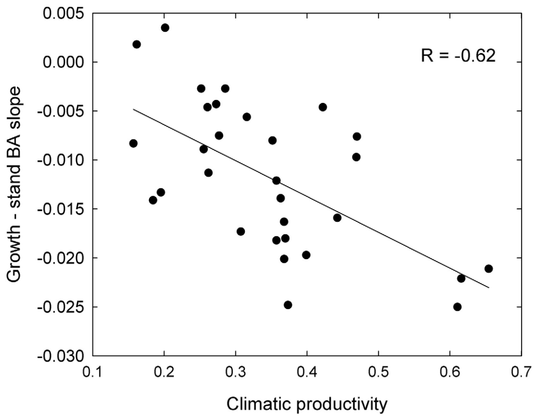

FIGURE 8 | Relationship between growth sensitivity to stand BA (growth—stand BA slope) and climatic productivity, for eucalypts <30 cm diameter.

(Tng et al., 2012). Following fire, there is prolific regeneration from seed, leading to intense competition for light, which causes self-thinning and drives rapid height growth (Gilbert, 1959; Ashton and Turner, 1979). These trends are apparent in the rapid growth decrease in small trees in response to increasing competition (**Figure 7**). A similar strong growth response by young trees to the basal area of neighboring larger trees has been interpreted as showing competition for light is more important than belowground competition in the initial successional phase of moist tropical forest (Van Breugel et al., 2012). By contrast, growth of *Eucalyptus fibrosa*, which grows in open forests in drier, warmer areas of eastern Australia, showed very little response to either tree size or RBA, probably because trees were smaller and more widely spaced (average stand BA was 15 m^2 ha^{-1}, cf. 44 m^2 ha^{-1} for *E. regnans*), so that competition for light was much less intense (**Figure 7**).

To conclude, we have used our continental-scale dataset to demonstrate that inter-tree competition and tree size strongly modify the effects of climate on growth rates of individual eucalypts. Therefore, when examining growth rates over such a macroclimatic gradient, it is crucial to consider interactions between climate, tree size, and the competitive environment, in addition to the main effects. These interactions provided evidence of strong, size-asymmetric competition in the productive environments, but little effect of competition in the least productive environments. We also showed that when using stand BA as a proxy for competition across broad productivity gradients, it should be relativized to reflect site productivity. However, stand BA is a useful proxy for competition at the local scale, and as a measure of competition for light.

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AUTHOR CONTRIBUTIONS

David M. J. S. Bowman conceived the project. Lynda D. Prior analyzed the data and wrote the paper. David M. J. S. Bowman contributed ideas to and significantly improved the manuscript.

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Diverse urban plantings managed with sufficient resource availability can increase plant productivity and arthropod diversity

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Buildings structures and surfaces are explicitly being used to grow plants, and these "urban plantings" are generally designed for aesthetic value. Urban plantings also have the potential to contribute significant "ecological values" by increasing urban habitat for animals such as arthropods and by increasing plant productivity. In this study, we evaluated how the provision of these additional ecological values is affected by plant species richness; the availability of essential resources for plants, such as water, light, space; and soil characteristics. We sampled 33 plantings located on the exterior of three buildings in the urban center of Brisbane, Australia (subtropical climatic region) over 2, 6 week sampling periods characterized by different temperature and rainfall conditions. Plant cover was estimated as a surrogate for productivity as destructive sampling of biomass was not possible. We measured weekly light levels (photosynthetically active radiation), plant CO_2 assimilation, soil CO_2 efflux, and arthropod diversity. Differences in plant cover were best explained by a three-way interaction of plant species richness, management water regime and sampling period. As the richness of plant species increased in a planter, productivity and total arthropod richness also increased significantly—likely due to greater habitat heterogeneity and quality. Overall we found urban plantings can provide additional ecological values if essential resources are maintained within a planter such as water, light and soil temperature. Diverse urban plantings that are managed with these principles in mind can contribute to the attraction of diverse arthropod communities, and lead to increased plant productivity within a dense urban context.

Keywords: urban biodiversity, ecosystem functions, ecosystem services, plant diversity, arthropod diversity, plant CO_2

INTRODUCTION

Rapid human concentration in cities, predicted to increase to 70% by 2030 (Unfpa, 2011), have led to great changes to ecosystems that erode biodiversity that in turn alters ecological processes vital to human well-being (Millennium Ecosystem Assessment, 2005). Ecosystem services are the benefits humans derive from ecosystem functions, e.g., carbon dioxide (CO_2) gas exchange and nutrient cycling that drive greenhouse gas regulation via plant growth services (Chapin et al., 1996). Studies have found that ecosystem services are regulated by both the diversity (Naeem et al., 1995; Tilman et al., 1996) and identity (Hooper et al., 2005) of the plant and animal species living in a community, making it vital to address the issue of declining biodiversity in our cities (Kendal et al., 2012). Climate change impacts and loss of habitat for biodiversity are two key challenges that could be mitigated through better management of urban biodiversity (Davies et al., 2011).

Despite clear evidence that bio-diverse and healthy ecosystems are beneficial to human health and wellbeing (Costanza et al., 1997), it is only recently that biodiversity has been considered when designing buildings (Daily, 1997). Roofs and walls of buildings can be used to grow plants including traditionally styled planter boxes designed and built integrally into the building structure. These "urban plantings" (see **Box 1** for generic definitions) represent ecologically underutilized space that could be transformed into green space (Dunnett and Kingsbury, 2004). Previous studies have focused on infrastructure and engineering related benefits that urban plantings can provide such as temperature reduction (Alexandri and Jones, 2008) and stormwater runoff reduction (Getter et al., 2007), but few studies have examined the use of urban plantings to mitigate climate change through CO_2 sequestration and to provide refuge habitat for biodiversity (Hooper and Vitousek, 1998; Oberndorfer et al., 2007; Cook-Patton et al., 2011). To address these two key knowledge gaps, our study evaluates how the plants, soils and habitat provision of urban plantings change with plant species richness and resource availability.

A substantial proportion of the CO_2 emissions produced in cities originates from buildings (Newman, 2006), yet buildings can also help reduce atmospheric CO_2 by incorporating plants

Box 1 | Definitions of key terms and concepts.

Urban plantings describe any type of vegetated building surface or structure, such as green roofs, green walls, green facades using raised planter and trellis system and also traditionally styled planter boxes designed and built integrally into a building's structure.

Green roof refers to any horizontal building space such as a rooftop or podium that has been partially or completely covered in several layers including waterproofing, drainage, soil substrate and vegetation. **Intensive green roofs** are essentially rooftop gardens, with greater than 150 mm substrate depth and require high maintenance. They are usually accessible, and are designed for aesthetic or recreational purposes, much like a regular garden. Intensive green roofs usually need to be incorporated into the building design, due to the weight bearing issues of substrate and vegetation. **Extensive green roofs** consist of shallow substrates of 50–150 mm depth and require little to no maintenance. They are usually inaccessible as they are primarily designed to provide environmental benefits. Extensive green roofs are relatively light-weight therefore they can usually be retrofitted to existing building rooftops.

Ecosystem functions are the physical, chemical, and biological processes or attributes that contribute to the self-maintenance of an ecosystem; in other words, what the ecosystem does.

Ecosystem services are the beneficial outcomes humans derive from ecosystem functions.

Diversity is a general term that can be defined at multiple levels and encompasses variation within and among species.

Richness refers to the number of species or genotype present in an assemblage, but does not describe the differences among these units.

and soils into their design via urban plantings. Urban plantings are highly managed systems, and generally have controlled and known abiotic factors such as water, soil composition, and age. Plant diversity may have a significant influence on productivity, and subsequently CO_2 uptake (Davies et al., 2011) and greater plant species diversity has been shown to produce greater carbon sequestration in grassland experiments (Tilman et al., 2006). Urban plantings have similar restricted growing conditions to green roofs. A recent study in Michigan (Whittinghill et al., 2014) examined carbon sequestration of three different types of green roofs of varying complexity and suggested that plant biomass and more complex plant communities increase the amount of carbon sequestered. However, to date, no green roof or urban planter study has specifically examined and tested the influence of plant diversity on carbon sequestration.

Urban plantings can also provide habitat and food for a variety of organisms and contribute to increased biodiversity in cities. Urban plantings may have greater importance as long-term habitats for smaller organisms such as arthropods due to the loss of their original habitats—many of these arthropods require specific micro-habitats to maintain viable populations (Gaston et al., 1998). Arthropods sampled in urban plantings have been shown to improve ecosystem function by contributing to soil forming processes (Schrader and Böning, 2006; Rumble and Gange, 2013), controlling pest insects (Hunter and Hunter, 2008), and pollinating plants (Hunter, 2002; McKinney, 2008; Colla et al., 2009). A study by Schindler et al. (2011) using pitfall traps found arthropod species richness to increase with increased vegetation cover on green roofs. Similarly, a recent study by Madre et al. (2013) found a positive relationship between arthropod species richness and plant species richness, using a standardized hand sampling method on the ground level, and within vegetation layers.

Increased density of cities means open green spaces are sometimes substituted with small plantings that are incorporated into a building. In this study, we measure plant diversity in relationship to how leaf CO_2 assimilation, soil CO_2 respiration, and arthropod diversity vary with plant species richness and resource availability across 33 plantings positioned around the exteriors of three buildings that are found within a 3 km radius of the CBD of Brisbane to address the following questions:

(1) Does either plant cover (a surrogate for productivity) vary with plant species richness, planter position and size, and resource availability (i.e., light and water)?
(2) Does plant CO_2 assimilation vary with plant richness, planter position and size, and resource availability (i.e., light and water)?
(3) Does soil CO_2 efflux vary with plant species richness, planter position and size, and resource availability (i.e., light and water)?
(4) Does arthropod diversity in urban plantings vary with plant species richness, planter position and size, and resource availability (i.e., light and water)?
(5) How does motility and accessibility influence the arthropod diversity found?

Measurements showing positive results from our initial queries above would indicate that urban plantings can support healthy biodiversity under managed microclimatic conditions, and therefore could provide additional ecological values in a dense urban environment.

MATERIALS AND METHODS

SITE DESCRIPTION

This study was conducted on three building sites in Brisbane, Queensland (**Figure 1**). Building three was situated closest to the city center (1 km), followed by building one (1.9 km) and building two (2.7 km). Two periods of sampling (hereafter referred to as sampling times) were performed between July and September 2013, over two seasons; winter and spring. The winter sampling time ran for 7 weeks, and the spring sampling period for 6 weeks. Building sites consisted of between 6 and 55 individual concrete planters built integrally within the building itself ranging in size from 1.5 × 0.35 × 0.4 m to 8.0 × 8.0 × 1.0 m (length × width × depth), which were all exposed to outside elements, i.e., light and rainfall, and were also managed with additional automatic drip-fed watering systems. A range of planters in each building were

FIGURE 1 | Photographs of the three building sites, labeled as 1–3 used in this study to show that all plantings were outside the building.

sampled to ensure that plantings with diverse characteristics were included in the study, for example light availability, distance from ground level, and area. A summary of sites and planter characteristics is available in **Table 1**, and specific sampling information for each site and sub-site, is available in Table S1 in the supplementary material. Site building plans of each site were used to determine planter area, depth, and distance from ground level. Information regarding building age, watering regime (measured as liters per 1 m^2 per week), and soil properties was obtained directly from site managers. Seasonal variations of cloud cover (eighths), humidity (%), precipitation (mm), and air temperature (°C) were recorded for each day of sampling, as these climactic variables may impact plant, and arthropod communities (Kremen et al., 1993; Geider et al., 2001). Climate data for the area encompassing the three study sites was sourced from the Bureau of Meteorology Brisbane weather station (Australian Government, 2013).

Throughout the 3 months of these experiments, sampling was always conducted between 9.00 am and 12.00 pm because these were the times when climatic conditions are most suitable for plants to be photosynthesizing. This is also a measure of control to compare processes across sampling times.

PLANT COVER AND PLANT CO_2 ASSIMILATION (SURROGATES FOR PRODUCTIVITY) MEASUREMENTS

Plant cover was estimated visually as percent cover for each planter using a modified Daubenmire method (Daubenmire, 1959) This method was used because it involves predicting the cover of all species within a plot, thus giving us a possibility of multiple layers of vegetation and therefore, values of cover above 100%. Plant cover is a commonly used estimate measure of plant primary productivity when destructive sampling is not possible (Röttgermann et al., 2000), and all plants were identified to species level (see Table S2 in the supplementary information for a detailed list of the plant species recorded).

Plant photosynthetic flux (μmol m^{-2} s^{-1}) was measured as a proxy of plant CO_2 assimilation rate (Hesketh and Baker, 1967). Leaf measurements were made *in situ* with an LI-6400 portable photosynthesis system, fitted with a leaf-chamber infrared gas analyser (LI-COR Inc.). Light information was measured with each individual leaf sample using the leaf-chamber's built-in light sensor. This sensor measured photosynthetically active radiation (hereafter PAR), a term which denotes the range of light wavelengths that can be used by green plants to photosynthesize. This method was used to gain a realistic indication of the rate of photosynthesis occurring within each of the sampled plantings. Individual plant leaves used in these measurements were tested at a concentration of 400 ppm CO_2, as this is the average ambient CO_2 level. CO_2 flux measurements were then logged when photosynthetic activity stabilized. These measurements were taken on two plant leaves from randomly selected individual plants from the two dominant understory, and two dominant mid-story plant species of each planter. In plantings with fewer than four plant species present, measurements were made on the next highest number present. The same individual plants were sampled for both sampling times. Leaves from the dominant top-story plants were inaccessible and therefore, not included in this study for three reasons: (1) sampling was a health and safety risk, (2) they were absent from the majority of plantings (81.82%), and (3) even when present they provided substantially less total cover (7.8%) in comparison to the combined midstorey and understorey plants. For more information regarding plant photosynthetic sampling, refer to Table S1.

SOIL CO_2 EFFLUX MEASUREMENTS

Soil CO_2 efflux (μmol m^{-2} s^{-1}) was measured as a proxy of soil CO_2 respiration rate (Donelan and Drennan, 1995). Measurements were made with an LI-6400 portable photosynthesis system (LI-COR Inc.), fitted with a 6400-09-soil CO_2 flux chamber and temperature probe. Ambient CO_2 concentration at the soil surface was recorded with the soil CO_2 flux chamber. A probe was used to record soil temperature. The soil chamber was then inserted to a depth of 2 cm, where CO_2 efflux based on the ambient concentration and soil temperature was

Table 1 | Summary of sites and planter characteristics including number of plantings sampled number sampled (total number present), soil properties, age, water regime, and soil depth information was obtained from interviews with building managers.

Characteristic	Building one	Building two	Building three
Number of plantings	12 (49)	15 (56)	6 (7)
Soil properties	Low organic content, loam and sand with hoop pine mulch	Low organic content, loam and sand with hoop pine mulch	Low organic content, loam and sand with tea tree mulch
Application of mulch	Once during initial planting phase	Once during initial planting phase	Once during initial planting phase
Watering regime (l/m^2/week: Mean ± Standard deviation)	15.33 ± 0.44	15.71 ± 0.37	71.4 ± 0
Age (years)	4	3	1
Soil depth range (cm)	40–75	40–75	100
Area range (m^2)	1–15	1–85	40–128
Distance to ground level range (m)	6.96–24.99	0–16.56	8–16
Distance to nearest green space (m)	50	25	40
Planter % vegetation cover (Mean ± Standard deviation)	59.8 ± 27.4	69 ± 19.8	143.5 ± 27.7
Plant richness (Mean ± Standard deviation)	4.1 ± 3.4	5.0 ± 0.9	10.5 ± 2.3

computed. This measurement was cycled automatically for three iterations, and the final measurement logged. The average soil CO_2 flux value based on these measurements was then calculated. These measurements were taken in each planting at randomly selected points every 10 m^2. One single measurement was taken for individual plantings under 10 m^2.

ARTHROPOD DIVERSITY MEASUREMENTS

To account for the presence of a wide range of arthropods across the different areas they inhabit, and also to assess arthropod dispersal, three different sampling methods were used: soil sampling, flight intercept traps, and direct sampling from plants. Morphospecies, which are species distinguished from others based on morphology, was used as a surrogate for identifying arthropods (Oliver and Beattie, 1996). To assess differing dispersal capabilities, arthropods were categorized as either winged or wingless. For a summary of arthropod species richness and abundance see Table S3.

Soil core samples were taken from each planter to provide an indication of soil dwelling arthropods. The soil core used in this study had a 54 mm diameter, and a depth of 100 mm. One sample was taken at a randomly selected point per 10 m^2 of each planter. One single sample was taken for individual plantings under 10 m^2. Arthropods in these soil samples were extracted with Tullgren funnels for 5 days (MacFadyen, 1953), with a 4 mm sieve (Upton, 1991). Specimens were preserved in propylene glycol before being sorted to morphospecies using a dissecting microscope at 100x magnification.

Sticky aphid/whitefly traps from Seabright Laboratories (Seabright Laboratories, 2013) were used to provide an indication of flying arthropods in each planter. Each trap used in this study was 16 × 10.2 cm in area, and reverse folded to expose an adhesive surface of bright yellow coloration. This specific hue of bright yellow is design to attract a wide-range of pest insects (Seabright Laboratories, 2013). Each trap was attached to a bamboo frame and suspended approximately 15 cm from the ground. The assembled traps were placed in each planter at randomly selected points every 10 m^2. One single trap was placed in individual plantings under 10 m^2. Sticky traps were present for the first 2 weeks of each sampling time, before being collected, and absent for the remainder of the sampling time. Collected sticky traps were stored in a fridge until sorted to morphospecies using a dissecting microscope at 100x magnification.

Standardized visual inspection was used to provide an indication of plant-dwelling arthropods (Gotelli et al., in press). Observations were made on selected individual plants, from each of the two dominant mid-story and two dominant understory plant species in each planter. We standardized this process by spending 10 min on each plant. Arthropods were provisionally identified to morphospecies on-site using a 10x hand lens, or were preserved in propylene glycol before being identified in the lab using a dissecting microscope at 100x magnification.

DATA ANALYSES

We used linear mixed effects models (hereafter LMEMs) to analyse the effects of different abiotic and biotic variables on plant cover, plant CO_2 assimilation rates, soil CO_2 respiration rates and arthropod diversity. Models were set-up with random effects of planter nested within building and the fixed effects tested were plant species richness, water regime, establishment age, and planter size. The base unit of measurement used was individual plantings nested within each site. We used diagnostic plots to check model assumptions (Pinheiro and Bates, 2000). There was no evidence of correlation of observations within groups in any of the models so we assumed that within groups, errors were normally distributed. Finally, we used Wald tests to assess the significance of terms in the fixed effects part of the models (Pinheiro and Bates, 2000). We changed the order of fixed effects in the model structure to check if order affected the significance of relationships and we found no effect. The statistical program R version 3.0.2 (R Development Core Team, 2012) and the package nlme (nonlinear mixed-effects) were used for these analyses.

RESULTS

TEMPERATURE AND RAINFALL DIFFERENCES BETWEEN SAMPLING PERIODS

On average, the winter sampling period had higher humidity, precipitation and cloud cover, and lower temperatures than the spring sampling period for each week of the study (**Figure 2**). Significant differences were found between the two seasons for cloud cover [$F_{(1,\ 28)} = 6.15$, $p = 0.02$], humidity [$F_{(1,\ 28)} = 10.41$, $p = < 0.01$], and air temperature [$F_{(1,\ 28)} = 14.58$, $p = < 0.01$], although the difference between season and precipitation were not significant [$F_{(1,\ 28)} = 2.07$, $p = 0.16$].

RELATIONSHIP BETWEEN PLANT COVER, PLANT RICHNESS, AND RESOURCE AVAILABILITY IN URBAN PLANTINGS

Plant species richness, management watering regimes and sampling time were found to be significant predictors of variation in plant cover in a three way interaction [$F_{(1,\ 227)} = 18.09$, $p < 1 \times 10^{-4}$; **Table 2**]. A positive relationship between plant species richness and plant cover was found [$F_{(1,\ 29)} = 37.99$, $p = 0.01$; **Figure 3**]. Plant cover varied marginally between winter and spring (**Figure 3**) because of the subtropical climate of the study sites. A positive relationship between irrigation and plant cover was also found [$F_{(1,\ 29)} = 40.67, p < 1 \times 10^{-4}$; **Figure 3**]. PAR, CO_2 assimilation rate, soil depth, area or establishment age were not significantly correlated with plant cover.

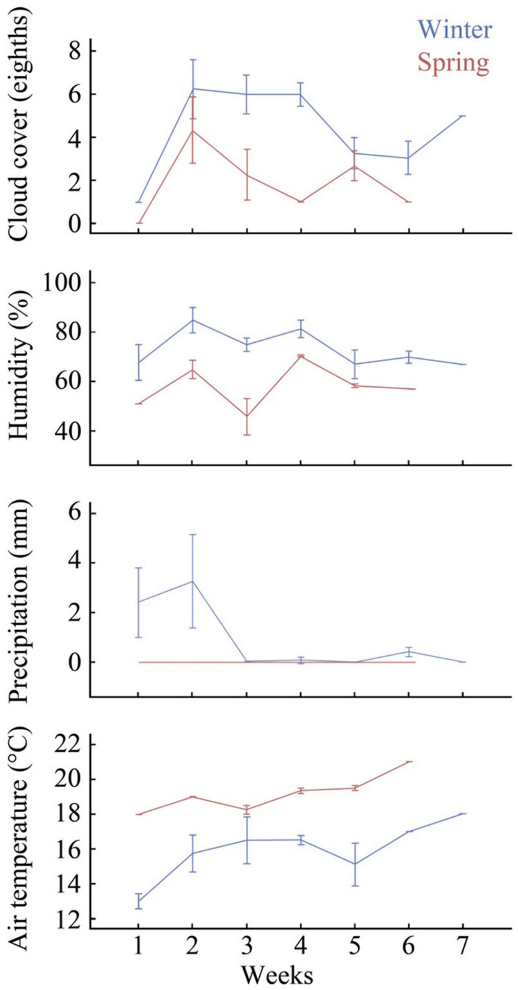

FIGURE 2 | Summary of climatic variables showing weekly average cloud cover (eighths), humidity (%), precipitation (mm), and air temperature (°C). Error bars indicate 95% confidence intervals, for winter and spring sampling times (7 and 6 weeks respectively). Data sourced from the Bureau of Meteorology Brisbane weather station (Australian Government, 2013).

Plant species richness did not vary from winter to spring and ranged between 1 and 15 plant species per planter, with building three having the highest average (10.5 ± 2.3), followed by building two (5.0 ± 0.9), and building one (4.1 ± 3.4). Building three was found to have the overall highest plant cover for both winter and spring (143.5 ± 27.7 and 152 ± 25.4; Figure S1) compared to building two (69 ± 19.8 and 70.4 ± 20.4; Figure S1) and building one (59.8 ± 27.4 and 60.2 ± 27.7; Figure S1). Building three received five times the amount of irrigation (71.4 l/m^2/week, **Table 1**).

RELATIONSHIP BETWEEN PLANT CO_2 ASSIMILATION RATE, PLANT RICHNESS, AND RESOURCE AVAILABILITY IN URBAN PLANTINGS

The relationship between plant species richness and plant CO_2 assimilation rate (**Figure 4**) was not significant [$F_{(1,\ 29)} = 0.64$, $p = 0.43$; **Table 3**]. PAR was found to have a strong significant positive relationship with plant CO_2 assimilation rates [$F_{(1,\ 230)} = 297.17$, $p < 1 \times 10^{-4}$; **Table 3**, **Figure 4**], where increased light correlated with increased plant CO_2 assimilation rates. Variation in plant CO_2 assimilation rates was not explained by sampling time, watering regime, soil depth, area, establishment age, or plant cover (**Table 3**).

Table 2 | Results from a Wald test of a linear mixed effect model with the response variable plant cover conducted to assess the significance of the fixed effects (i.e., PAR, CO_2 assimilation rate, sampling times, watering regime, soil depth, planter area, and establishment age).

Variables	numDF	denDF	*F*-value	*p*-value
Plant species richness	1	29	37.99	0.01
Establishment age	1	1	19.60	0.14
Area	1	1	27.98	0.12
Soil depth	1	29	0.08	0.78
Watering regime	1	29	40.67	$<1 \times 10^{-4}$
Sampling time	1	230	176.87	$<1 \times 10^{-4}$
CO_2 assimilation rate	1	230	0.71	0.40
PAR	1	230	1.24	0.27
Plant species richness: watering regime	1	27	0.73	0.40
Plant species richness: sampling time	1	227	382.98	$<1 \times 10^{-4}$
Watering regime: sampling time	1	227	146.96	$<1 \times 10^{-4}$
Plant species richness: watering regime: sampling time	1	227	18.09	$<1 \times 10^{-4}$

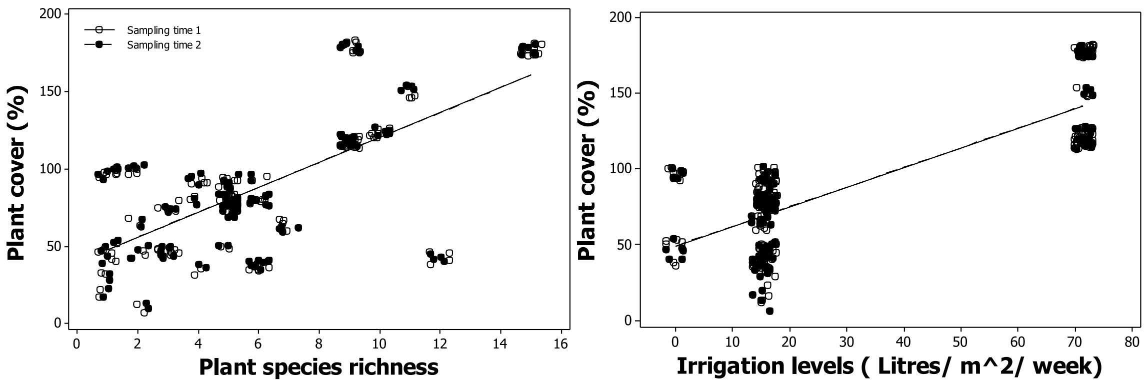

FIGURE 3 | Modeled relationship between plant cover (%), plant species richness and irrigation levels depending on the sampling time period.

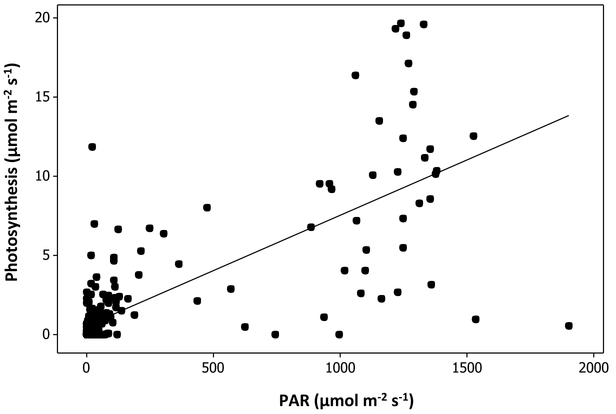

FIGURE 4 | Modeled relationship between plant CO_2 assimilation rates and photosynthetically active radiation (PAR) measurement taken from above of the leaves measured.

Table 3 | Results from a Wald test of a linear mixed effect model with the response variable assimilation rate to assess the significance of the fixed effects (i.e., PAR, sampling times, watering regime, soil depth, area, establishment age, plant species richness, and plant cover) on a linear mixed effects model for plant CO_2 assimilation rate.

Variables	numDF	denDF	*F*-value	*p*-value
Plant cover	1	230	1.49	0.23
Plant species richness	1	29	0.64	0.43
Establishment age	1	1	5.30	0.26
Area	1	1	15.71	0.16
Soil depth	1	29	0.38	0.54
Watering regime	1	29	1.31	0.26
Sampling time	1	230	1.19	0.28
PAR	1	230	296.17	$<1 \times 10^{-4}$

RELATIONSHIP BETWEEN SOIL CO_2 EFFLUX, PLANT RICHNESS, AND RESOURCE AVAILABILITY IN URBAN PLANTINGS

No significant relationship was found between plant species richness and soil CO_2 respiration [$F_{(1, 29)} = 0.38, p = 0.54$; **Table 4**]. Soil temperature was found to have a significant positive relationship with soil CO_2 respiration [$F_{(1, 235} = 217.48, p < 0.01$; **Table 4**, **Figure 5**]. Variation in soil CO_2 respiration rates could not be explained by sampling times, establishment age, area, soil depth, or watering regime (**Table 4**).

RELATIONSHIP BETWEEN TOTAL ARTHROPOD RICHNESS, PLANT RICHNESS, AND RESOURCE AVAILABILITY IN URBAN PLANTINGS

A significant positive relationship was found between total arthropod species richness and plant species richness [$F_{(1, 29)} = 6.04, p = 0.02$; **Table 5**, **Figure 6**], soil depth [$F_{(1, 29)} = 11.71, p < 1.9 \times 10^{-3}$; **Table 5**, **Figure 6**], and watering regime [$F_{(1, 29)} = 27.64, p < 1 \times 10^{-4}$; **Table 5**, **Figure 6**]. Total arthropod richness was higher during second "spring" sampling time period [$F_{(1, 230)} = 69.02, p = < 1 \times 10^{-4}$; **Table 5**; **Figure 6**], with plant species richness, soil depth, and watering regime each showing a significant two-way interaction with sampling time (**Table 5**). Variation in total arthropod species richness was not explained by establishment age, area of the planter, distance to the ground, or distance to the nearest green space (**Table 5**).

All arthropods sampled in our study were classified as either winged or wingless to provide an indication of arthropod motility and therefore colonization of urban plantings. We found both winged [$F_{(1, 29)} = 6.09, p < 0.02$; Table S1, Figure S2], and wingless [$F_{(1, 29)} = 3.90, p < 0.05$; Table S1, Figure S2] arthropod species richness positively correlated with plant species richness suggesting that arthropod community richness were not dependent purely on flight capability, and may therefore have a different origin.

DISCUSSION

Arthropod richness and plant productivity increased with plant richness and increased resource availability, showing that urban plantings can provide additional ecological values. Plant management practices were found to strongly influence the overall productivity of the plantings including light and water availability. Surprisingly, we found that neither planting age, nor planter distance from the ground were important predictors of arthropod richness or productivity. Overall, our findings are a promising result for the design and maintenance of urban plantings as we

Table 4 | Results from a Wald test of a linear mixed effect model with the response variable soil CO_2 respiration to assess the significance of the fixed effects (i.e., PAR, sampling times, water regime, planter depth, planter area, establishment age, soil arthropod species richness, soil arthropod species abundance, plant species richness, and plant coverage) on a linear mixed effects model for soil CO_2 respiration.

Variables	numDF	denDF	*F*-value	*p*-value
Plant cover	1	29	0.39	0.54
Plant species richness	1	29	0.38	0.54
Age	1	1	0.29	0.69
Area	1	30	0.06	0.80
Soil depth	1	29	0.09	0.76
Watering regime	1	29	1.12	0.30
Sampling times	1	235	1.44	0.23
Soil temperature	1	235	217.48	$<1 \times 10^{-4}$

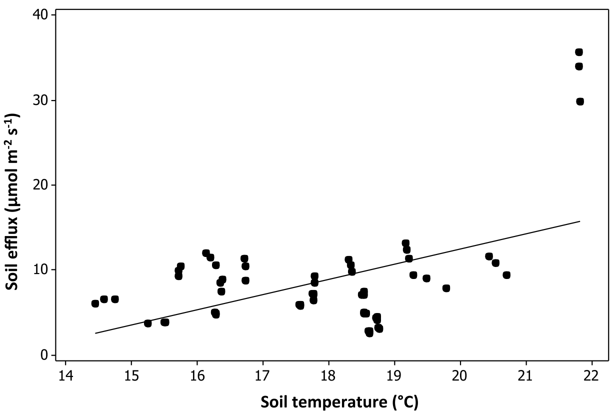

FIGURE 5 | Modeled relationship between soil CO_2 respiration and soil temperature.

found a few easily managed characteristics can improve the productivity of urban plantings, i.e., the number of plant species, water, light availability, and soil properties.

Productivity was influenced by plant species richness, water, and climate in urban plantings. Ecological theory predicts that plant diversity can enhance productivity (e.g., plant biomass and overall energy flow), which in turn is linked to a wide range of ecosystem services, such as the assimilation of carbon (Waide et al., 1999). In our study, we found that plant diversity and plant cover had a significant positive relationship, but we also found that water regimes (and season) had a significant positive relationship with plant cover. The plantings with the highest species richness also received the highest levels of irrigation; therefore, water treatments are likely confounded with the plant diversity fixed effects. Surprisingly, other factors controlled by management practices such as establishment age, planter area (total area), soil depth, and PAR, did not have a significant relationship with plant cover.

Plant CO_2 assimilation rates were influenced by PAR in urban plantings. Plant photosynthesis limits the amount of carbon that can be created and stored within ecosystems (Dias et al., 2010); and studies have found a positive relationship between plant diversity and carbon assimilation in natural systems (Conti and Díaz, 2013). Only two studies have examined green roof carbon sequestration potential, and although their findings suggest that greater plant biomass, and more complex plant communities increase CO_2 uptake, the impact of plant diversity of this important service remains unknown (Getter et al., 2009; Whittinghill et al., 2014).

Contrary to previous studies, we found that neither plant species richness, nor plant cover have significant relationships with CO_2 assimilation rates. Instead, and not surprisingly PAR was found to significantly influence CO_2 assimilation rate, suggesting that other key requirements of plant productivity such as planter characteristics, and sampling times were not limiting factors.

SOIL CO_2 EFFLUX IS INFLUENCED BY SOIL TEMPERATURE IN URBAN PLANTINGS

Soil respiration is driven by autotrophic and heterotrophic respiration (Daily, 1997). The richness, amount of plant litter, and root exudates can influence soil respiration by altering soil chemistry. We found no significant relationship between either plant species richness and soil CO_2 efflux, or plant cover and soil CO_2 efflux; instead soil temperature was found to influence soil CO_2 efflux.

Our result suggests that soil respiration within urban plantings may not be limited by either the amount, or the variety of dead plant material and plant root exudates. Instead, urban planting soil respiration may be due to the amount of soil organic matter derived from the pre-installed mulch and soils, although we did not test this here. If this is the case, plant diversity and productivity may not matter to soil respiration within urban plantings because the availability of soil organic matter is not a limiting factor. However, the plantings we investigated were between 1 and 6 years in age, so it is possible that plant diversity, and productivity may matter more in older plantings.

In our study, soil temperature was found to be positively correlated with soil CO_2 efflux. Soil temperature is a critical limiting factor of soil organisms, especially microbes, which are the major contributors of all soil respiration (Yiqi and Zhou, 2010). Surprisingly, other environmental variables known to influence soil respiration such as age or water (Curiel Yuste et al., 2007), did not appear to have an influence. Landscapes accumulate more leaf litter, microbes and root biomass as they age (Matamala et al., 2008), yet age may not have been important as the plantings in our study were relatively young, ranging from 1 to 6 years, and this may be limiting soil forming processes (Schrader and Böning, 2006; Pavao-Zuckerman, 2008). Irrigation can either assist or inhibit the rate of plant litter decomposition by microbes depending on its availability (Curiel Yuste et al., 2007). In our study, irrigation was not significantly correlated with soil CO_2 efflux.

Arthropod richness was influenced by plant species richness, water, and climate in urban plantings. Countless ecological studies of natural habitat have found plant richness, both amount and quality, influence arthropod richness (Siemann et al., 1998; Haddad et al., 2009). In city buildings, we also found both plant richness positively correlated with total arthropod richness

Table 5 | Results from a Wald test of a linear mixed effect model with the response variable total species richness to assess the significance of the fixed effects (i.e., sampling times, distance to green space, distance to ground level, water regime, soil depth, planter area, establishment age, plant cover and plant species richness).

Variables	numDF	denDF	*F*-value	*p*-value
Plant species richness	1	29	6.04	0.02
Plant cover	1	230	675.41	$<1 \times 10^{-4}$
Establishment age	1	1	36.58	0.10
Area	1	1	97.28	0.06
Soil depth	1	29	10.11	3.5×10^{-3}
Watering regime	1	29	27.64	$<1 \times 10^{-4}$
Distance to ground level	1	230	0.90	0.34
Distance to nearest green space	1	1	0.004	0.96
Sampling time	1	230	69.02	$<1 \times 10^{-4}$
Plant species richness: depth	1	27	8.75	6.4×10^{-3}
Plant species richness: water	1	27	0.96	0.34
Plant species richness: sampling time	1	229	118.11	$<1 \times 10^{-4}$
Depth: water	1	27	1.06	0.31
Depth: sampling time	1	229	119.70	$<1 \times 10^{-4}$
Water: sampling time	1	229	330.32	$<1 \times 10^{-4}$

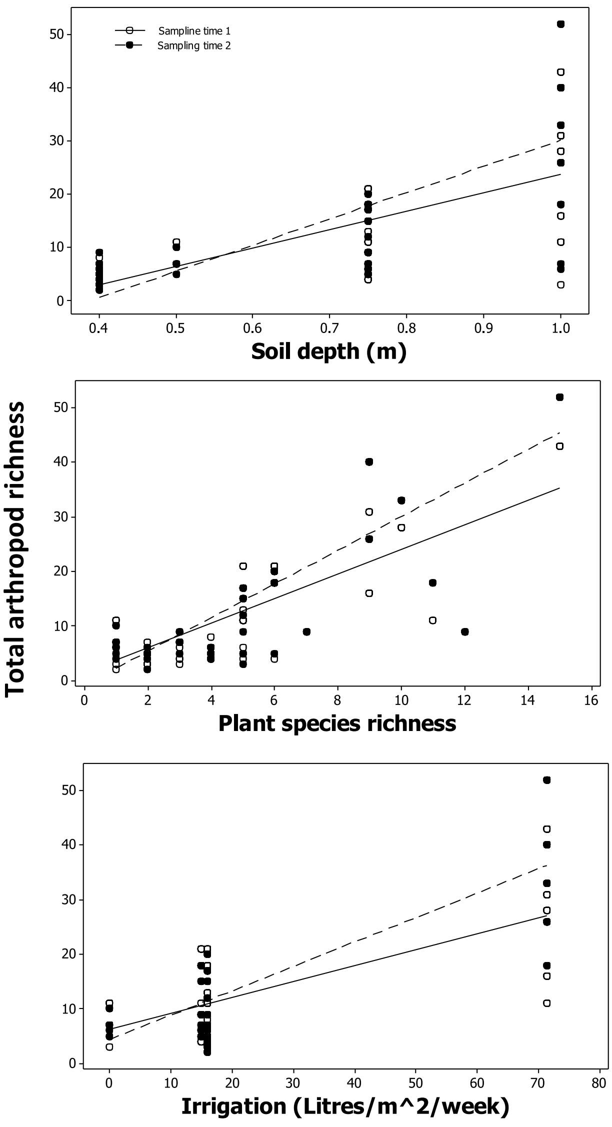

FIGURE 6 | Modeled relationship between total arthropod richness, soil depth, plant species richness, and irrigation levels.

on urban plantings, with plant richness having the stronger relationship. This result supports other studies that found a positive correlation between plant richness and invertebrate diversity in urban areas (Smith et al., 2006; Nielsen et al., 2014).

Even in a subtropical climate, season was found to influence total arthropod species richness, and the arthropod species richness of the three sampling methods. This finding is not surprising given that arthropods are more active due to the higher temperatures associated with seasonal change. The soil depth and the watering regime used by each respective building manager were correlated with arthropod species richness. Water is an essential resource requirement of many arthropod species, and was also found to be positively correlated with plant cover (Verhoef and Selm, 1983; Rumble and Gange, 2013). The positive correlation between soil depth and total arthropod richness is likely explained by more soil containing more resources, and niche space for arthropods (Byrne, 2007).

A fundamental understanding of how arthropods colonize urban plantings is needed if urban plantings are important as habitats for arthropods. Horizontal distance from the building site to the nearest green space was found in our study to have no influence on arthropod species richness or abundance, regardless of sampling method or motility classification. Some green roof studies have shown that the proximity of surrounding green space habitats has a positive effect on urban arthropod communities (Penone et al., 2012; Vergnes et al., 2012); however, other studies have shown surrounding habitats has no effect on arthropod richness at all (Schindler et al., 2011; Madre et al., 2013).

Urban plantings with diverse plant species may be able to support diverse arthropod communities, even if the plantings themselves are small and isolated from other habitats. Our study found that vertical distance from the plantings to the ground level had no influence on any measure of arthropod species richness or abundance. This provides a possible explanation as to why both, higher winged and wingless arthropod richness, were found in plantings with higher plant species richness, and supports the findings of Macivor and Lundholm (2011), who found a similar result in a comparative arthropod study between green roofs and adjacent level-ground habitats.

The species-area relationship is considered one of the strongest general theories in ecology (Huston, 1979), as it consistently holds across ecosystems. Although the sizes of urban plantings in our study were highly variable, ranging from 1 to 128 m^2, area was only found to influence wingless arthropod species richness, and sticky trap arthropod richness. The sticky trap method was the only method used that is active—attracting arthropods; therefore, dispersal ability may again be a key explanation.

CONCLUSIONS

Overall we found that the application of just a few easily managed characteristics of urban plantings such as plant species richness, access to light, water levels, and soil temperature can make a difference to the overall productivity of the plants and the arthropod diversity attracted. We measured pre-existing urban plantings that were managed differently, which was both a benefit and limitation of our study particularly since essential conditions for increased plant productivity such as water availability were highest for the plantings that had the highest species richness (although richness varied in the multiple plantings in each building). The benefit of measuring established urban plantings was that we were able to capture the natural variability between buildings and climatic conditions and therefore, were able to quantify more realistic trends than in a controlled experiment. Despite some confounding conditions, we found evidence that plant species richness and resource availability were strong drivers of arthropod richness and plant productivity.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fpls.2014.00517/abstract

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Competition for nitrogen between *Fagus sylvatica* and *Acer pseudoplatanus* seedlings depends on soil nitrogen availability

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Competition for nitrogen (N), particularly in resource-limited habitats, might be avoided by different N acquisition strategies of plants. In our study, we investigated whether slow-growing European beech and fast-growing sycamore maple seedlings avoid competition for growth-limiting N by different N uptake patterns and the potential alteration by soil N availability in a microcosm experiment. We quantified growth and biomass indices, ^{15}N uptake capacity and N pools in the fine roots. Overall, growth indices, N acquisition and N pools in the fine roots were influenced by species-specific competition depending on soil N availability. With inter-specific competition, growth of sycamore maple reduced regardless of soil N supply, whereas beech only showed reduced growth when N was limited. Both species responded to inter-specific competition by alteration of N pools in the fine roots; however, sycamore maple showed a stronger response compared to beech for almost all N pools in roots, except for structural N at low soil N availability. Beech generally preferred organic N acquisition while sycamore maple took up more inorganic N. Furthermore, with inter-specific competition, beech had an enhanced organic N uptake capacity, while in sycamore maple inorganic N uptake capacity was impaired by the presence of beech. Although sycamore maple could tolerate the suboptimal conditions at the cost of reduced growth, our study indicates its reduced competitive ability for N compared to beech.

Keywords: growth strategies, inorganic N uptake, inter-specific competition, intra-specific competition, N pools, organic N uptake, specific amino acids

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Introduction

Plant species have evolved different strategies to maximize plant survival and reproduction by various combinations of physiological and morphological traits, depending on the environmental conditions (Reich et al., 1997, 2003; Craine et al., 2009). Many studies have investigated these different combinations of plant functional traits over the past decades with the focus on leaf traits and/or seed production (Reich et al., 2003), but knowledge on root traits is still scarce (Craine et al., 2009). Fast-growing species tend to have a higher photosynthetic capacity because of their higher light-capture area deployment per unit mass [high specific leaf area (SLA)] and faster turnover of plant parts, thus allowing flexibility in the plant's response to the spatial heterogeneity of the environment (Reich et al., 1997; Westoby et al., 2002). This, in turn, ensures short-term advantages over slow-growing plants (Grime et al., 1994; Westoby et al., 2002), particularly in resource-limited environments in which competition is high. Thus, competition for resources, especially the growth-limiting macronutrient nitrogen, constitutes a major challenge for plants, including not only competition with soil microorganisms (Dannenmann et al., 2009; Rennenberg et al., 2009), but also other vegetation components, such as herbaceous and/or woody species (Fotelli et al., 2002, 2005; Simon et al., 2010b, 2011, 2014).

In Central Europe, European beech (*Fagus sylvatica*) represents the dominant tree species of the potential natural vegetation in moist to moderately dry areas of the submountainous altitude range (Diekmann, 1996). Beech is favored by forest practitioners and governments because nowadays forest management practices have changed from supporting conifer monocultures to the preference of mixed species stands thereby promoting the natural regeneration of deciduous tree species (Fotelli et al., 2001, 2004; Petritan et al., 2009). The survival and growth of beech regeneration depends mainly on the ability to co-exist with highly competitive species (Tognetti et al., 1998; Fotelli et al., 2001, 2002, 2004, 2005). For example, Fotelli et al. (2002) showed that inorganic N uptake capacity of slow-growing beech seedlings was significantly reduced when grown together with the fast-growing pioneer shrub *Rubus fruticosus* and decreased even further with drought stress (Fotelli et al., 2004). Furthermore, other studies investigated the competition for nitrogen between beech and other potential competitors, such as soil microorganisms (Dannenmann et al., 2009), other tree species (Simon et al., 2010b, 2014), or even different developmental stages within a species (Simon et al., 2011). Beech seedlings and adult beech trees, for example, avoided competition for N by seasonal timing of N acquisition (Simon et al., 2011). Furthermore, in short-term studies investigating the competition for N between beech and sycamore maple seedlings (Simon et al., 2010b, 2014), we found evidence for different N uptake strategies that might depend on the growth strategies of the species.

Sycamore maple (*Acer pseudoplatanus*) – a relatively fast-growing species compared to slow-growing beech – might require large amounts of N by root uptake to meet its resource requirements for growth and development (Poorter et al., 2012). Because sycamore maple shares the spectrum where beech is dominant on calcareous substrate (Ellenberg, 1996), the two species might have evolved different strategies to successfully compete for nitrogen (Simon et al., 2010b) or avoid competition (Simon et al., 2011, 2014). Simon et al. (2010b) found that short-term competition between seedlings of both species lead to a reduced inorganic and organic N uptake capacity by slow-growing beech with limiting soil N, whereas inorganic N uptake capacity by fast-growing sycamore maple increased significantly. Under reduced light conditions, N acquisition by sycamore maple seedlings was negatively affected in the presence of beech indicating that beech is optimally attuned to shade conditions and outcompetes sycamore maple at least in short-term competition with reduced light availability (Simon et al., 2014). However, in these studies the focus was on short-term consequences (i.e., competing for 4 days), but not the implications of competition between two woody species when grown together for several months, a time during which competition might result in more distinct strategies of N uptake.

Therefore, this study aimed to elucidate (1) whether European beech and sycamore maple avoid competition for growth limiting N by different N uptake strategies, and (2) whether these strategies are altered by soil N availability. For this purpose, seedlings of European beech and sycamore maple were grown under controlled conditions in a microcosm experiment in which growth and biomass indices, N uptake capacity by the fine roots and N pools (i.e., total N, structural N, soluble protein-N, total amino acid-N, as well as specific amino acid-N) in the fine roots were analyzed. To ensure that differences in N uptake capacity were due to the other species, we set up the microorganisms also as intra-specific controls, i.e., with several individuals of the same species. Levels of specific amino acid-N in the roots were quantified to investigate overall patterns of potential differences in specific amino acid-N in the roots with competition and N supply.

Materials and Methods

Plant Material and Growth Conditions

Mycorrhizal seedlings of *F. sylvatica* L. (provenance Swabian Alb) and *A. pseudoplatanus* L. (provenance "Sueddeutsches Huegel- und Bergland montane Stufe") of similar height (0.25–0.5 m) and structural characteristics were purchased from a commercial tree nursery (Schlegel & Co. Gartenprodukte GmbH, Riedlingen, Germany). One-year-old seedlings were chosen for this study, because the early developmental stage of seedlings is crucial for seedling establishment, in particular under competition for limited resources with other species (Madsen and Larsen, 1997; Zerbe, 2002). Microcosms (355 mm × 255 mm × 315 mm) were filled with a homogenous mixture of 0.7–1.2 mm silica sand (1 part), 0.1–0.5 mm silica sand (18 parts), perlite (19 parts), and torf (2 parts; Floragard Vertriebs GmbH für Gartenbau, Oldenburg, Germany) to keep plant available N from the soil substrate at a minimum. Seedlings were planted into the microcosms in November 2010 (see Experimental Design), over-wintered outside, and were transferred back to the greenhouse at the end of March 2011. Until the start of the experiment in mid-April, all microcosms were sufficiently irrigated every second day with an artificial low N solution (see below). Plants were grown under 16/8 h day/night conditions until the final harvest. Seedlings received natural daylight plus an artificial daylight supplied by mercury lamps (SON-T AGRO 400, Philips GmbH, Eindhoven, The Netherlands) with an average illumination intensity at canopy level of 412 ± 32 μmol m^{-2} s^{-1} (mean ± SD) during the day representing a tree-fall gap light environment (Tognetti et al., 1998). The average air temperatures were 21.1 ± 6.7 and 17.7 ± 3.8°C (day/night, mean ± SD). The average relative humidity was 41.5 ± 14.5% and 47.7 ± 7.1% (day/night, mean ± SD).

Experimental Design

The experiment had a 3 × 2 factorial design with three levels of competitive interference (i.e., beech grown in intra-specific competition, sycamore maple grown in intra-specific competition, and beech and sycamore maple grown in inter-specific competition) and two levels of nitrogen supply (i.e., low or high) resulting in six treatment combinations. The level of competition was defined as: (1) European beech only (eight beech seedlings, BB), (2) sycamore maple only (eight sycamore maple seedlings, MM), and (3) beech and sycamore maple growing in competition (four beech plus 4 sycamore maple seedlings, BM). For each treatment, ten replicate microcosms were used containing eight seedlings planted with a tree to tree distance of c. 85 mm. This distance is well within the distance for neighboring plants to compete for resources (Gaudet and Keddy, 1988; Nernberg and Dale, 1997; Imo and Timmer, 1999). Two rows of three seedlings were arranged along each side and two seedlings were planted in the middle row of each microcosm. In microcosms containing both species, seedlings were spaced alternating by species to ensure that each individual was surrounded by individuals of the other species. Overall, the single species microcosms were used to study the effect of intra-specific competition and served as a control for the inter-specific competition. From the mixed species microcosms, both species were harvested and analyzed for inter-specific competition. This is shown in the results as BB-B (intra-specifically-grown beech), BM-B (beech grown in competition with sycamore maple), MM-M (intra-specifically grown sycamore maple), and BM-M (sycamore maple grown in competition with beech). BM-B and BM-M originate essentially from the same planting design, but for comparison purposes, a distinction is made whether the impact of sycamore maple on beech or beech on sycamore maple is being considered. After leaf development (mid-April), microcosms were separated into high/low N supply treatments and irrigated with 1 L of either low N (with a total of 151 μM N) or high N (with a total of 550 μM N) artificial nutrient solution every second day until the end of the experiment. The artificial low N solution was based on the soil solution at a low soil N field site in the Swabian Alb (Dannenmann et al., 2009) containing 100 μM KNO_3, 90 μM $CaCl_2*2H_2O$, 70 μM $MgCl_2*6H_2O$, 50 μM KCl, 24 μM $MnCl_2*4H_2O$, 20 μM NaCl, 10 μM $AlCl_3$, 7 μM $FeSO_4*7H_2O$, 6 μM K_2HPO_4, 1 μM NH_4Cl, as well as the amino acids glutamine and arginine (25 μM each) at pH 6.5. The artificial high N solution was based on the soil solution of a high soil N field site in the Bavarian alpine upland containing 20 μM $Al_2(SO_4)_3$, 75 μM $CaCl_2 \cdot 2H_2O$, 4 μM $FeCl_3 \cdot 6H_2O$, 14 μM KCl, 10 μM $MnCl_2 \cdot 4H_2O$, 40 μM $MgCl_2 \cdot 6H_2O$, 4.5 μM Na_2HPO_4, 20 μM NaCl, 50 μM NH_4Cl, 300 μM KNO_3, 100 μM glutamine, and 100 μM arginine at pH 4.7 (Stoelken et al., 2010). Glutamine and arginine were chosen as the most abundant amino acids in beech roots (Gessler et al., 1998).

Harvest and Sample Preparation

Before the start of the experiment in mid-April, an initial harvest was conducted sampling three microcosms of each competition regime to determine the initial biomass and leaf area of seedlings required for calculation of relative growth rates (RGRs; see below). Ten weeks later, the final harvest was performed subsequent to ^{15}N uptake experiments. At the initial and final harvest, seedlings were separated into fine roots, coarse roots, stems, and leaves which were oven-dried over 48 h at 65°C. Fresh and dry weights were determined. Leaf area was measured using an area meter (ΔT area meter, Delta-T devices, London, UK). In addition, fine root samples were shock-frozen in liquid nitrogen (after determining the fresh weight) and transferred to −80°C for storage until further processing. Prior to N metabolite quantification, frozen tissue was finely ground in liquid nitrogen.

Growth and Biomass Allocation Indices

Average RGR was calculated for each seedling as $RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1)$, where W_1 is the total plant biomass (g dw) per individual at the initial harvest of the experiment at t_1 (day of the initial harvest), W_2 is the total plant biomass (g dw) per individual at t_2 (day of the final harvest; Grubb et al., 1996; Simon et al., 2010a). W_1 was calculated from the average biomass of seedlings of each species ($n = 24$ grown in intra-specific competition, $n = 16$ grown in inter-specific competition). For other growth indices, data from t_2 was used, including SLA, leaf mass ratio (LMR), and leaf area ratio (LAR, total leaf area as a proportion of total plant biomass). Net assimilation rate (NAR) was calculated by dividing RGR by LAR. Leaf nitrogen productivity (LNP, an index of plant growth relative to leaf N) was determined according to: $LNP = RGR/(N_a * LAR)$, where N_a is the foliar nitrogen concentration per unit leaf area (Simon et al., 2010b). Furthermore, root:shoot ratio ($R: S_m$) was determined on a mass basis.

^{15}N Uptake Experiments

For the ^{15}N uptake experiments, the two center seedlings and in addition the middle one of each site from the inter-specific competition microcosms were chosen. The ^{15}N enrichment technique as described by Gessler et al. (1998) and Simon et al. (2010b) was applied to determine N uptake capacity. Both long sides of the microcosm were cut open for easy access to the fine roots. Fine roots still attached to the plants were carefully dug out and rinsed with distilled water to remove adhering substrate particles. To quantify N uptake rates, roots were incubated for 2 h (between 10:00 am to 14:00 pm to avoid diurnal variation in N uptake (Gessler et al., 2002) in 4 mL of either low or high N solution (see above) with one of four N compounds labeled either as $^{15}NO_3^-$, $^{15}NH_4^+$, or $^{15}N/^{13}C$ double-labeled glutamine or arginine, or control solutions without ^{15}N label (to account for the natural abundance of ^{15}N in the fine roots). After 2 h incubation, the submersed root tips and moistened upper parts (∼8–10 mm) were cut off, washed twice with 0.5 μM $CaCl_{2,}$ dried out with cellulose paper and oven-dried for 48 h at 65°C. Fresh and dry weight was determined.

Quantification of ^{15}N, ^{13}C, and Total N Amounts in Fine Roots and Leaves

For the determination of ^{15}N and ^{13}C abundance and total N in fine roots and leaves, the dried tissue (48 h, 60°C) was ground into a fine homogeneous powder using a ball mill. Aliquots of 1.2–2 mg were transferred into tin capsules (IVA Analysentechnik, Meerbusch, Germany) and analyzed using an elemental analyzer (NA2500, CE Instruments, Milan, Italy), coupled via a Conflo II interface to an isotope ratio mass spectrometer (Delta Plus, Thermo Finnigan MAT GmbH, Bremen, Germany). Working standards (glutamic acid), calibrated against the primary standards USGS 40 (Glutamic acid, $\delta^{13}C_{PDB} = -26.39$) and USGS 41 (Glutamic acid, $\delta^{13}C_{PDB} = 37.63$) for $\delta^{13}C$ and USGS 25 (ammonium sulfate, $\delta^{15}N_{Air} = -30.4$) and USGS 41 ($\delta^{15}N_{Air} = 47.600$) for $\delta^{15}N$, were analyzed after every 12th sample to detect a potential instrument drift over time.

N uptake capacity (nmol g^{-1} fw h^{-1}) was calculated based on the incorporation of ^{15}N into fine roots and the respective plant biomass according to the equation by Geßler et al. (1998): N uptake capacity = $((^{15}N_l\text{-}^{15}N_c)^*N_{tot}^*dw^*10^5)/(MW^*fw^*t)$, where $^{15}N_l$ and $^{15}N_c$ are the atom% of ^{15}N in labeled (N_l) and control plants (N_c, natural abundance), respectively, N_{tot} is the total N percentage, and MW is the molecular weight (^{15}N g mol^{-1}), t represents the incubation time (120 min). Based on ^{13}C incorporation into the root fresh weight, the uptake rates of amino acids were generally lower compared to those based on the ^{15}N incorporation indicating either the degradation of amino acids in the incubation solution or on the root surface, or respiration of amino acid-derived carbon inside the roots (Simon et al., 2011).

Quantification of Total Soluble Protein in Fine Roots

Total soluble proteins in fine roots were quantified according to the method by Dannenmann et al. (2009). Frozen fine ground roots (c. 50 mg) were extracted in 1 mL extraction buffer (50 mM Tris-Cl (pH 8.0), 1 mM EDTA, 15% glycerol (v:v), 1 mM phenylmenthylsulfonyl fluoride, 5 mM dithiothreitol, 0.1% Triton X-100). After 30 min incubation on the shaker at 4°C followed by centrifugation at 14,000 *g* for 10 min at 4°C, 500 μL trichloroacetic acid (10%) were added to 500 μL aliquots of the supernatant, and then incubated for 10 min at room temperature. After centrifugation at 14,000 *g* for 10 min, the supernatant was carefully discarded and the protein pellets were dissolved in 0.5 mL 1 M KOH. Bradford reagent (1 mL; Amresco Inc., Solon, OH, USA) was added to 50 μL aliquots of the extracts for quantification of total soluble protein. After 10 min of incubation at room temperature in the dark, the optical density was measured in a UV-DU650 spectrophotometer (Beckman Coulter Inc., Fullerton, CA, USA) at 595 nm. Bovine serum albumin (BSA, sigma A-6918) was used as a standard.

Quantification of Total and Specific Amino Acids and Ammonium in Fine Roots

Amino acids and ammonium were extracted according to the method of Winter et al. (1992). Aliquots of c. 50 mg frozen, homogenized root tissue were extracted in 0.2 mL buffer (20 mM Hepes, 50 mM EGTA, 10 mM NaF, pH 7.0) and 1 mL methanol: chloroform (3.5:1.5, v:v). After shaking for 30 min at 4°C, 600 μL distilled H_2O were added to the samples, mixed, and centrifuged for 5 min at 4°C. This extraction step was repeated once. The quantification of total amino acid was determined from the combined supernatants according to the method by Liu et al. (2005). For quantification of total amino acids, aliquots (100 μL) of the supernatant and 100 μL of ninhydrin reagent a 50:50 (v:v) mixture of solution A (containing 4.2 g citric acid·H_2O, 0.16 g $SnCl_2 \cdot 2H_2O$, and 40 mL 1 M NaOH, made up to 100 mL with distilled water at pH 5) and solution B containing 4 g ninhydrin in 100 mL ethylene glycol monomethyl ether) were boiled at 95°C for 30 min. Isopropanol (1.25 mL, 50%) was added to the mixture followed by 15 min incubation. The optical density was measured using a DV-UV650 spectrophotometer (Beckman Coulter Inc., Fullerton, CA, USA) at 570 nm. Glutamine was used as a standard. For quantification of specific amino acids and ammonium, the extracts were shock-frozen in liquid $N_{2,}$ and freeze-dried for 96 h. The composition and concentration of amino compounds and ammonium was determined in 50 μL extracts analyzed with a Water Acquity UPLC-System (Waters Corp., Milford, MA, USA) using a modified standard protocol (using an AccQ-Tag^{TM} Ultra column 2.1 mm × 100 mm, 1.7 μm, 0.7 mL/min flow, column temperature 61°C) as previously described (Luo et al., 2009). Amino acid Standard H (#NCI0180, Pierce Biotechnology, Inc., Rockford, IL, USA) was used as an analytical standard plus additional specific amino acids and ammonium added (with 2.5 μmol in 0.1 N HCl each) according to the composition of the analyzed sample.

Quantification of Nitrate in Fine Roots

Nitrate was quantified according to the method described by (Dannenmann et al., 2009). Approximately 100 mg washed polyvinylpyrrolidone (PVPP, Sigma-Aldrich Inc., Steinheim, Germany) were soaked in 1 mL distilled H_2O overnight. About 50 mg root material was added to the solution and shaken for 1 h in the dark. Samples were boiled at 95°C for 10 min, followed by 10 min centrifugation at 4°C. Aliquots of 150 μL supernatant were measured in an auto-sampler (AS3500, Thermo Separation Products, Piscataway, NJ, USA) connected with an ion chromatography system (DX120, Dionex, Idstein, Germany). The ion chromatography system was equipped with a guard column (RFIC^{TM} IonPac AS9-SC, 4 mm × 250 mm, Dionex, Idstein, Germany), an analytical column (IonPac AS9-SC, 4 mm × 250 mm, Dionex, Idstein, Germany) and a self-regenerating suppressor (ASRS-ULTRA II, 4 mm, Dionex, Idstein, Germany). An eluent solution of 2.0 mM sodium carbonate and 0.75 mM sodium bicarbonate was used for the separation of different anions. An anion mixture of NO_3^-, PO_4^{3-}, SO_3^{2-}, and SO_4^{2-} was used as a standard.

Statistical Analyses

For all measured parameters, normality tests and Levene's test for homogeneity of variances were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). To detect differences among treatments, two-way analyses of variance (ANOVAs) were conducted using SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, USA). Two factors were defined as (1) high/low N supply and (2) competition regimes. Holm-Sidak *post hoc* test was performed subsequently to compare differences within each factor by SigmaPlot (Systat Software Inc., San Jose, CA, USA). Datasets of specific amino acids were subjected to principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) using MetaboAnalyst 2.0 (Xia et al., 2012).

Results

Combined Influence of Soil N Availability and Competition Regime on Growth Indices, N Acquisition and N Pools

To study the combined influence of soil N availability and competition regime on growth indices, N acquisition and N pools in the fine roots of beech and sycamore maple seedlings, two-way ANOVAs were performed. Only R:S$_m$, LAR, foliar N$_a$, ammonium, and Arg-N uptake capacity ($P \leq 0.030$), as well as nitrate-N and soluble protein-N pools in the fine roots ($P \leq 0.023$) were significantly affected by the combination of both treatments (**Table 1**).

Consequences of Soil N Availability on N Acquisition and N Pools in Both Species

In European beech seedlings, decreasing soil N availability (i.e., high vs. low N supply) resulted in significant changes in N uptake capacity and N pools in the fine roots in both competition regimes (**Table 1**). No significant differences with N supply were found for any of the growth indices except for an increase in foliar N$_a$ in beech regardless of competition regime with decreasing soil N supply ($P<0.001$; **Tables 1** and **2**). Furthermore, decreasing soil N availability led to declining N uptake capacities for all four tested N sources regardless of competition regime ($P < 0.001$; **Table 1**; **Figure 1**). With regard to N pools in the fine roots of beech, only beech seedlings grown in inter-specific competition had higher levels of ammonium-N with low compared to high N supply ($P = 0.037$; **Figure 2**).

TABLE 1 | Two-way ANOVA analyses of growth indices, N uptake capacity and N pools in the fine roots of beech and sycamore maple seedlings.

	N supply * competition	N supply	Competition
Growth indices			
R:S$_m$	**0.018**	0.292	0.241
RGR (mg g^{-1} d^{-1})	0.600	0.101	**<0.001**
NAR (g m^2 d^{-1})	0.211	0.658	**<0.001**
LNP (g g(N$_m$)$^{-1}$ d^{-1})	0.212	0.058	**<0.001**
LMR (g g^{-1})	0.905	**0.016**	**<0.001**
LAR (cm^2 g^{-1})	**0.030**	0.186	**0.004**
SLA (m^2 g^{-1})	0.412	0.419	**<0.001**
N$_a$ (g m^{-2})	**<0.001**	**<0.001**	**<0.001**
N uptake capacity			
Ammonium	**<0.001**	**<0.001**	**<0.001**
Nitrate	0.496	**<0.001**	**<0.001**
Arginine-N	**<0.001**	**<0.001**	**<0.001**
Glutamine-N	0.172	**<0.001**	**<0.001**
N pools			
Total N	0.123	0.283	**<0.001**
Structural N	0.099	0.120	0.153
Total soluble protein-N	**0.023**	**<0.001**	**0.031**
Total amino acid-N	0.226	**<0.001**	**0.001**
Nitrate-N	**<0.001**	**<0.001**	**<0.001**
Ammonium-N	0.494	**0.004**	**<0.001**

The effects of all parameters were separated by two factors (1) N supply and (2) competition regimes. R:S$_m$, root/shoot mass ratio; RGR, relative growth rate; NAR, net assimilation rate; LNP, leaf nitrogen productivity; LMR, leaf mass ratio; LAR, leaf area ratio; SLA, specific leaf area; N$_a$, nitrogen concentration per unit leaf area. Bold values indicate the significance level of 0.050.

Similar to beech, N uptake capacity in sycamore maple generally declined with decreasing soil N supply ($P \leq 0.012$; **Figure 1**). Furthermore, with declining soil N availability, levels of nitrate-N (regardless of competition regime) and total amino acid-N (only with intra-specific competition) significantly decreased ($P \leq 0.001$), whereas levels of soluble protein-N (with intra-specific competition) and ammonium-N (with inter-specific competition) increased ($P \leq 0.040$; **Figure 2**) in sycamore maple. With regard to growth indices, LAR decreased ($P = 0.023$) and foliar N$_a$ increased ($P<0.001$) significantly with decreasing soil N supply in sycamore maple only with inter-specific competition.

Consequences of Competition Regime on Growth Indices, N Acquisition and N Pools in European Beech Seedlings

Beech seedlings responded to inter-specific competition with sycamore maple with regard to growth indices, N uptake capacity and N pools in the fine roots (**Table 2**; **Figures 1** and **2**). Beech showed no significant differences of biomass by the influence of sycamore maple under high N supply, whereas under low N supply RGR, NAR, and LNP decreased in presence of sycamore maple ($P \leq 0.032$; **Table 2**). Generally, for beech, organic N (i.e., Gln and Arg) was the preferred N source regardless of soil N availability and competition regime ($P \leq 0.001$; **Figure 1**). With high soil N availability, competition with sycamore maple only led to an increase in Gln-N and Arg-N uptake capacity ($P \leq 0.033$) in beech, but exhibited no changes in ammonium and nitrate uptake capacity (**Figure 1**) compared to intra-specific competition. With regard to N pools in beech roots, ammonium-N concentration in beech roots decreased when grown with sycamore maple with low N supply ($P = 0.044$), while no other changes in N pools in beech were found at high or low soil N availability (**Figure 2**).

Consequences of Competition Regime on Growth Indices, N Acquisition and N Pools in Sycamore Maple Seedlings

Similar to beech, sycamore maple seedlings responded with changes in N uptake capacity and N pools in the fine roots when grown in competition with beech, but also showed differences in growth and biomass indices. At high soil N availability, RGR, NAR, LNP, and foliar N$_a$ decreased was significantly in the presence of beech ($P \leq 0.008$; **Table 2**). Furthermore, inorganic N uptake capacity (i.e., nitrate, ammonium) were significantly lower in sycamore maple in the presence of beech ($P \leq 0.047$; **Figure 1**) except for ammonium with low N supply, whereas no changes were found for organic N uptake. With regard to N pools in the fine roots, concentrations of soluble protein-N and nitrate-N increased in sycamore maple in the presence of beech at high soil

TABLE 2 | Biomass and leaf parameters of beech and sycamore seedlings with high/low soil N supply (mean ± SD).

	High N supply				Low N supply			
	BB-B	**BM-B**	**BM-M**	**MM-M**	**BB-B**	**BM-B**	**BM-M**	**MM-M**
$R:S_m$	0.832 ± 0.109^{a}	0.891 ± 0.141^{a}	0.780 ± 0.195^{a}	0.874 ± 0.204^{a}	0.906 ± 0.171^{a}	0.873 ± 0.198^{a}	0.865 ± 0.173^{a}	0.813 ± 0.196^{a}
RGR (mg g^{-1} d^{-1})	6.232 ± 5.179^{b}	3.325 ± 6.559^{ab}	1.841 ± 6.303^{a}	12.685 ± 4.525^{c}	4.587 ± 7.278^{b}	0.866 ± 5.721^{a}	1.912 ± 6.559^{ab}	10.581 ± 5.724^{c}
NAR (g m^2 d^{-1})	0.178 ± 0.148^{b}	0.095 ± 0.148^{ab}	0.101 ± 0.26^{a}	0.417 ± 0.153^{c}	0.148 ± 0.235^{b}	0.027 ± 0.175^{a}	0.080 ± 0.269^{ab}	0.395 ± 0.214^{c}
LNP (g $g(N_m)^{-1}$ d^{-1})	1.667 ± 1.700^{b}	0.847 ± 0.995^{ab}	1.349 ± 2.751^{a}	4.932 ± 1.189^{c}	0.943 ± 0.985^{b}	0.014 ± 0.041^{a}	0.800 ± 1.936^{ab}	4.574 ± 2.051^{c}
LMR (g g^{-1})	0.170 ± 0.034^{b}	0.180 ± 0.036^{b}	0.106 ± 0.028^{a}	0.105 ± 0.021^{a}	0.162 ± 0.023^{b}	0.168 ± 0.021^{b}	0.102 ± 0.020^{a}	0.097 ± 0.020^{a}
LAR (cm^2 g^{-1})	34.937 ± 5.569^{a}	35.091 ± 23.746^{a}	$30.644 \pm 9.375^{a*}$	29.644 ± 6.925^{a}	30.913 ± 5.380^{a}	32.604 ± 6.806^{a}	$24.245 \pm 6.106^{a*}$	26.807 ± 9.093^{a}
SLA (m^2 g^{-1})	0.318 ± 0.027^{a}	0.334 ± 0.036^{a}	0.421 ± 0.127^{b}	0.399 ± 0.083^{b}	0.326 ± 0.032^{a}	0.327 ± 0.035^{a}	0.375 ± 0.095^{ab}	0.404 ± 0.036^{b}
N_a (g m^{-2})	$1.190 \pm 0.104^{b*}$	$1.201 \pm 0.077^{b*}$	$0.809 \pm 0.025^{a*}$	1.057 ± 0.158^{b}	$1.594 \pm 0.112^{c*}$	$1.408 \pm 0.120^{c*}$	$1.341 \pm 0.045^{b*}$	1.000 ± 0.105^{a}

$R:S_m$, root/shoot mass ratio; RGR, relative growth rate; NAR, net assimilation rate; LNP, leaf nitrogen productivity; LMR, leaf mass ratio; LAR, leaf area ratio; SLA, specific leaf area; N_a, nitrogen concentration per unit leaf area. BB-B: beech grown in intra-specific competition, BM-B: beech grown in inter-specific competition with sycamore maple, BM-M: sycamore maple grown in inter-specific competition with beech, MM-M: sycamore maple grown in intra-specific competition. Different small letters indicate significant differences between the competition regimes (i.e., BB-B, BM-B, BM-M, MM-M) within one soil N supply treatment ($P \leq 0.05$). Asterisks indicate significant differences between high and low soil N supply within one competition regime ($P \leq 0.05$).

N availability, while no other changes in N pools in beech were found ($P \leq 0.014$; **Figures 2C,E**).

At low soil N supply, sycamore maple had reduced RGR, NAR, and LNP ($P \leq 0.001$), whereas foliar N_a increased ($P \leq 0.001$) when grown in competition with beech (**Table 2**). Similar to high N supply, nitrate-N uptake capacity in sycamore maple decreased in the presence of beech. Concentrations of total N, soluble protein, total amino acids and ammonium N increased significantly in the fine roots of sycamore maple grown in competition with beech at low soil N supply ($P \leq 0.004$; **Figure 2**).

Comparing European Beech and Sycamore Maple Seedlings

The responses to inter/intra-specific competition with regard to growth and biomass indices, as well as N uptake capacity and N metabolites in the fine roots between the two species, differed depending on species and soil N supply. At intra-specific competition, beech had lower RGR, NAR, LNP, and SLA but higher LMR than sycamore maple independent of soil N availability ($P \leq 0.016$; **Table 2**). However, when beech and sycamore maple were grown in inter-specific competition, both species showed similar $R:S_m$, RGR, NAR, LNP, and LAR regardless of soil N supply. Comparing beech and sycamore maple in inter-specific competition, with high soil N supply, beech seedlings had a lower SLA and higher N_a and LMR ($P \leq 0.002$; **Table 2**), whereas at low soil N supply no significant differences were found for SLA between beech and sycamore maple in inter-specific competition.

Beech showed lower inorganic N uptake capacity than sycamore maple in intra-specific competition regardless of N supply, except for ammonium with low N supply ($P<0.001$; **Figure 1**). Beech and sycamore maple grown in inter-specific competition, showed similar inorganic N uptake, except for nitrate which was still higher in sycamore maple than in beech ($P = 0.019$; **Figure 1**). Regarding organic N uptake capacity, Arg-N uptake capacity was generally higher in beech compared to sycamore maple regardless of N availability and competitive regimes ($P \leq 0.004$), except for beech and sycamore maple in intra-specific competition with low N supply. With high N supply, Gln-N uptake capacity increased in beech in presence of sycamore maple.

With regard to N metabolites in the fine roots, beech seedlings had lower concentrations of total N (except for that in intra-specific competition at low N supply), nitrate-N (only at high N supply), total soluble protein-N, total amino-acid-N and ammonium-N (only in inter-specific competition; $P \leq 0.001$) than sycamore maple seedlings, regardless of soil N availability and competition regime (**Figure 2**).

Principal component analysis showed that competition regime and soil N availability led to overall changes in amino acid-N composition in the fine roots of beech and sycamore maple (**Figure 3**). With high N availability, the influence of intra-specific competition showed overlapping areas in beech and sycamore maple, whereas the presence of the competing species led to species-specific amino acid composition. This species specificity became more pronounced when N supply was limited, and both species showed similar composition regardless of competition regime.

Discussion

Response of Beech Seedlings to Competition for N Depends on Soil N Availability

For beech seedlings, the consequences of competition for N with sycamore maple had no direct influence on growth indices with

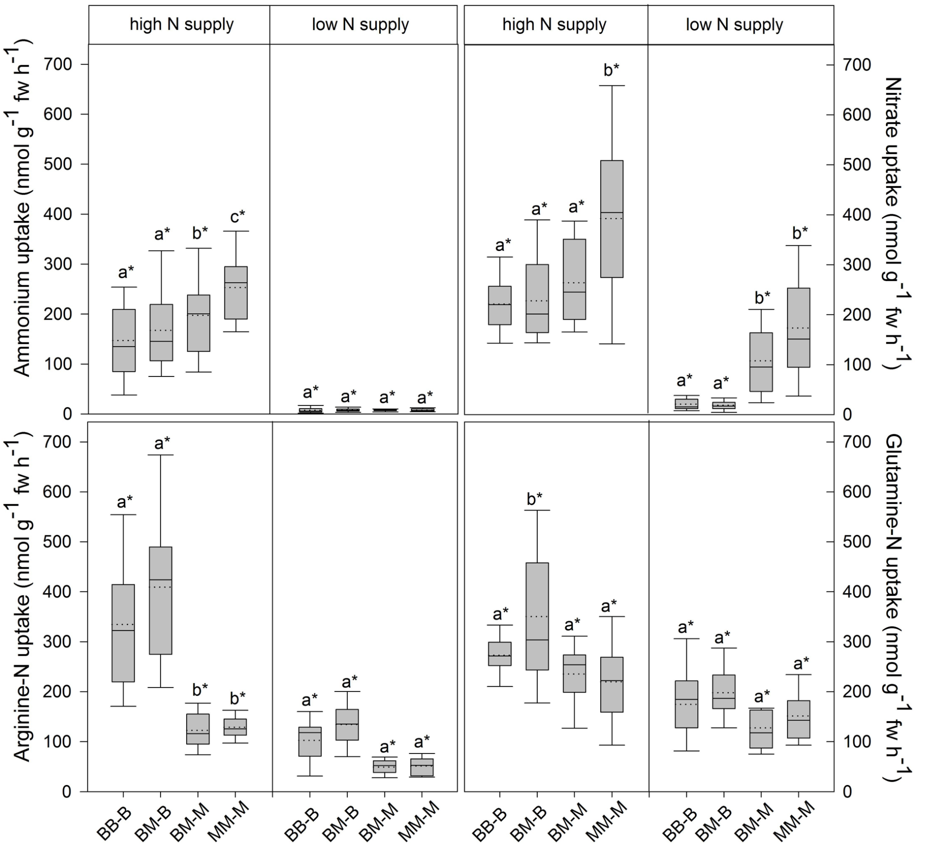

FIGURE 1 | Inorganic and organic N uptake capacity (nmol g^{-1} fw h^{-1}) in beech and sycamore maple seedlings with high or low soil N supply. BB-B: beech grown in intra-specific competition, BM-B: beech grown in competition with sycamore maple, BM-M: sycamore maple grown in competition with beech, MM-M: sycamore maple grown in intra-specific competition. Box plots show means (dotted lines) and medians (straight lines; $n = 14$ for each treatment). Different small letters indicate significant differences between the four competition regimes (i.e., BB-B, BM-B, BM-M, MM-M) within one soil N supply ($P \leq 0.050$). Asterisk indicates significant difference between high and low soil N supply within one competition regime ($P \leq 0.050$).

high N supply, but physiological changes were found regardless of soil N availability when comparing with intra-specifically grown beech seedlings. This indicates that the underlying mechanisms regulating growth patterns might shift depending on the competing species. Comparing the N acquisition strategies of beech seedlings grown in intra- vs. inter-specific competition, with high soil N availability, beech seedlings showed an increased Gln-N and Arg-N uptake capacity when competing with sycamore maple. This strategy is in contrast to previous results from a study investigating the consequences of short-term competition for N between beech and sycamore maple seedlings (Simon et al., 2010b). In this study, beech seedlings had significantly lower inorganic and organic N uptake capacity in the presence of maple which might have been due to the release of an inhibiting compound by sycamore maple (Simon et al., 2010b). A similar study using the same composition of N compounds applied to the roots and investigating the effect of light availability on the competition between beech and sycamore maple also under short-term conditions (Simon et al., 2014) could not show the inhibiting effect of sycamore maple on beech. Thus, it is still unclear, under what conditions inhibition of N uptake in beech seedlings might occur. The experimental set-up of the experiments conducted on short-term competition (Simon et al., 2010b, 2014) is not directly comparable with the present study. The results of our present study indicate that under close to natural conditions beech does not have a disadvantage, but might have rather adapted to the competition by increasing organic (i.e., Gln and Arg) N uptake. This preference of organic over inorganic N is consistent with other studies on beech roots (e.g., Dannenmann et al., 2009; Simon et al., 2010b, 2011, 2014). N pools in the fine roots of beech did not change in the presence of sycamore maple regardless of N supply, except for a decrease of ammonium-N concentration in beech in the presence of sycamore maple with limited N supply. However, we measured only N pools in fine roots, thus differences in levels of N metabolites might also be due to metabolite transport from above ground tissues (Herschbach et al., 2012). These changes in N acquisition and allocation to N pools in the fine roots indicate that different N use strategies of beech in the presence of sycamore maple depend on soil N supply. Furthermore, N acquisition of beech seedlings was adapted to the competition with sycamore maple regardless of N supply, because N uptake capacity of beech was not impaired.

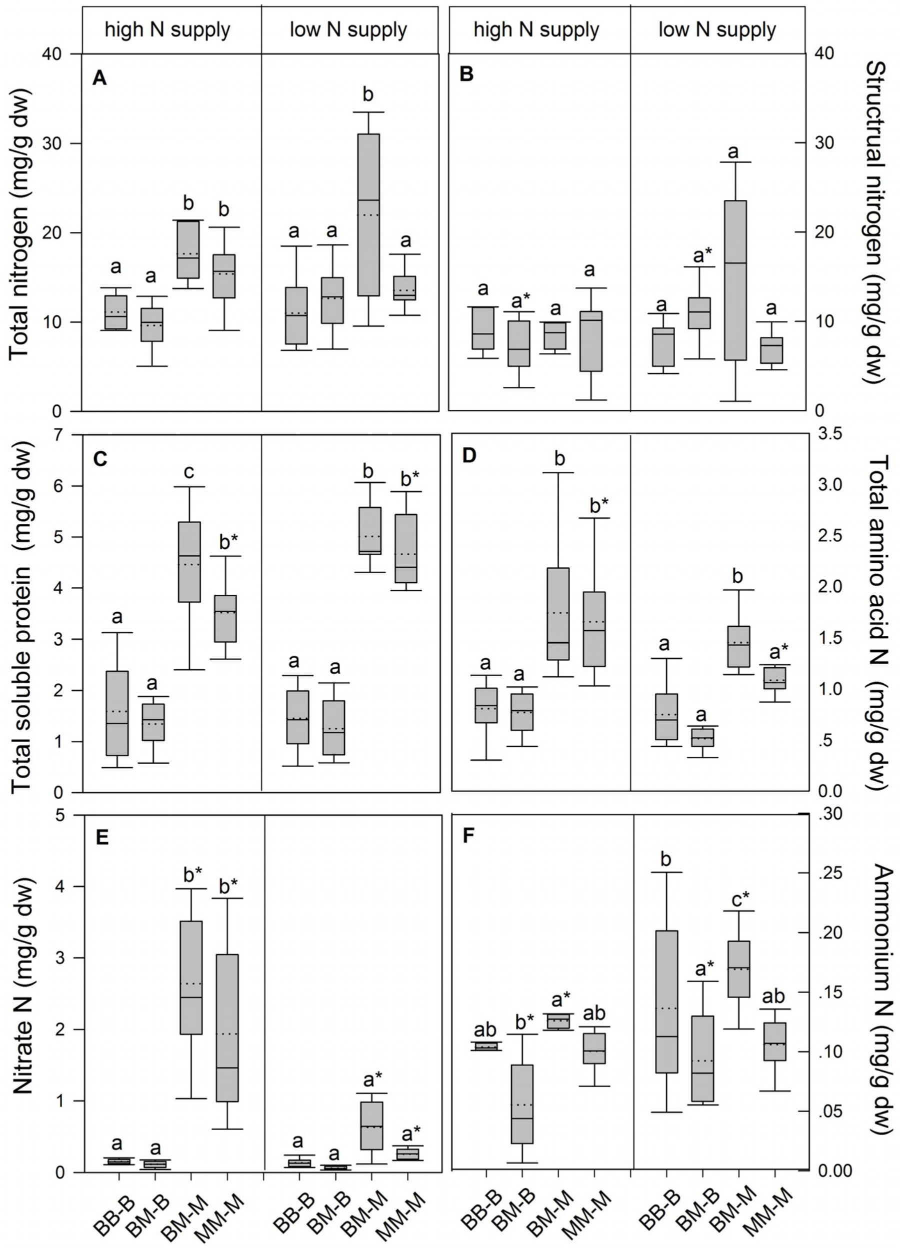

FIGURE 2 | Nitrogen pools (mg g^{-1} dw) in the fine roots of beech and sycamore maple seedlings with high or low soil N supply. (A) Total N, **(B)** structural N, **(C)** total soluble protein-N, **(D)** total amino acid-N, **(E)** nitrate-N, **(F)** ammonium-N. BB-B: beech grown in intra-specific competition, BM-B: beech grown in competition with sycamore maple, BM-M: sycamore maple grown in competition with beech, MM-M: sycamore maple grown in intra-specific competition. Box plots show means (dotted lines) and medians (straight lines; $n = 14$ for each treatment). Different small letters indicate significant differences between the competition regimes (i.e., BB-B, BM-B, BM-M, MM-M) within one soil N supply ($P \leq 0.050$). Asterisks indicate significant differences between high and low soil N supply within one competition regime ($P \leq 0.050$).

Response of Sycamore Maple Seedlings to Competition for N Varies with Competition Regime and Soil N Availability

For sycamore maple, competition led to a reduction in RGR due to lower LNP and NAR (both regardless of soil N availability) in sycamore maple seedling regardless of soil N supply, thus showing a visible response to competition with beech seedlings. The reduction in inorganic N acquisition by sycamore maple (regardless of N supply) indicates that sycamore maple might be outcompeted in competition by beech as was also indicated in the short-term competition with decreasing light availability (Simon et al., 2014). Apparently, N acquisition strategies of sycamore maple depend not solely on abiotic stressors but shift with abiotic–biotic stressor combination, e.g., decline in N uptake

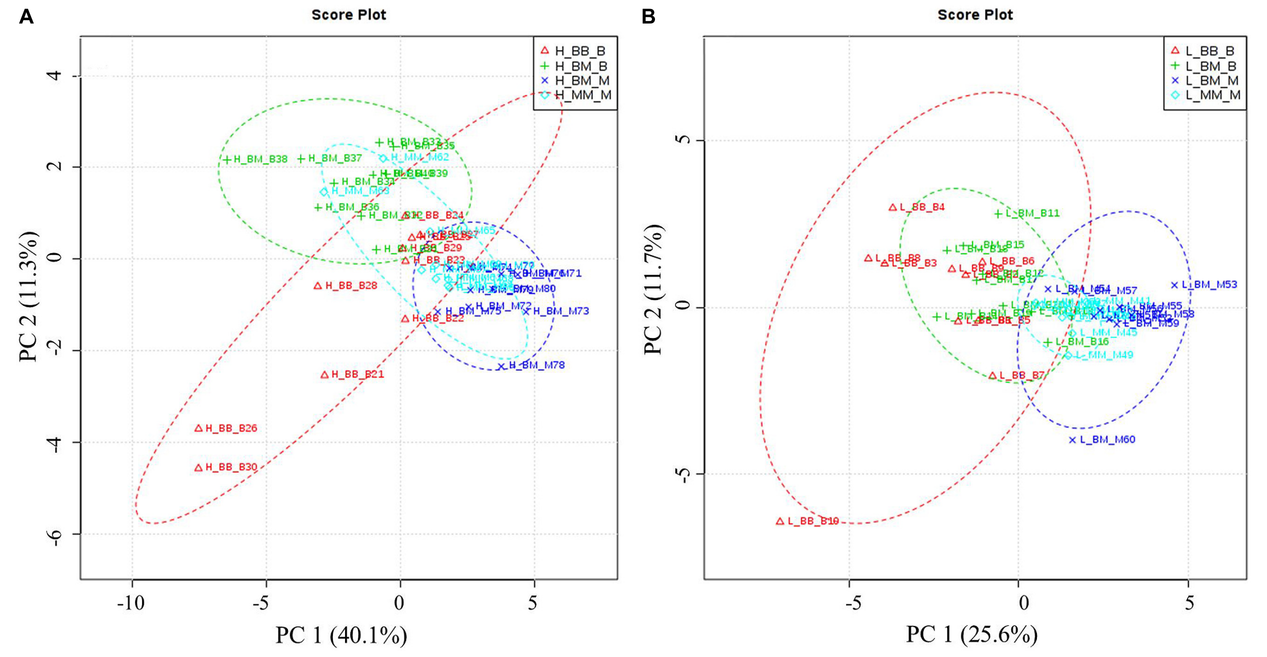

FIGURE 3 | Principal components analysis (PCA) based on specific amino acid concentration. H: with high soil N supply **(A)**, L: with low soil N supply **(B)**. BB-B: beech grown in intra-specific competition, BM-B: beech grown in competition with sycamore maple, BM-M: sycamore maple grown in competition with beech, MM-M: sycamore maple grown in intra-specific competition. **(A)** PCA of specific amino acids levels under high soil N supply. According to the variance of the specific amino acids along PC1 and 2 (capture 51.4% of the total variances), the groups of beech and maple under inter-specific competition were separated distinctly. **(B)** PCA of specific amino acids levels under low soil N supply. Beech and sycamore maple under inter-specific competition are grouped with a little part overlapped (PC1 and 2 explain 37.3% of variance).

capacity with reduced light availability in short-term competition (Simon et al., 2014), but regardless of N availability in competition (present study). Sycamore maple tended to prefer inorganic N (i.e., ammonium and nitrate) at high soil N availability regardless of competition regime in contrast to results found for beech seedlings (Stoelken et al., 2010). However, limited N availability also led to a general decrease in N uptake capacity as found in beech (Stoelken et al., 2010). In contrast to beech seedlings, total N, soluble protein-N, total amino acid-N, and ammonium-N concentrations in the fine roots of sycamore maple increased at low soil N availability when seedlings were grown in competition with beech suggesting that sycamore maple responded stronger to the change in soil N supply compared to beech. The levels of soluble protein-N in the fine roots of sycamore maple increased when grown in competition with beech confirming the results from previous experiments that sycamore maple – when grown in competition with beech (Simon et al., 2010b, 2014) – synthesizes proteins, probably representing a specific adaptation of sycamore maple.

Different Strategies of Competition – Beech vs. Sycamore Maple

Beech and sycamore maple seedlings showed different responses to soil N availability with regard to growth, N acquisition and composition of N pools in the fine roots, similar to different responses to changing light availability investigated in a previous study (Simon et al., 2014). Furthermore, the responses were also influenced by competition between the two species in the present study. When grown in intra-specific competition, beech and sycamore maple show different growth strategies at least at the seedling level. Beech is a relatively slow-growing, whereas sycamore maple is a relatively fast growing species (Ellenberg, 1996), represented in lower LNP, SLA, and NAR regardless of soil N availability. The species-specific differences in N acquisition, namely a higher organic N uptake capacity for beech seedlings than in sycamore maple, and a higher inorganic N uptake capacity in sycamore maple than in beech, confirm the theory that competition for N can be avoided (e.g., Simon et al., 2011; Hodge and Fitter, 2013). These contrasting results compared to previous studies investigating the competition for N between beech and sycamore maple grown in short-term competition (Simon et al., 2010b, 2014) indicate that N uptake strategies might shift over time. Whereas in short-term competition, plant species might actually compete for limited resources, in the longer run they acclimate to the conditions and develop an avoidance strategy. Species differed also in their N pools in the fine roots regardless of soil N availability and competition regime. Sycamore maple had generally higher levels of total N, total soluble protein-N, total amino acid-N and nitrate-N compared to beech. Furthermore, the different approaches to cope with low soil N availability (i.e., differences in N allocation to fine root N pools) suggest that beech is better adapted to N limitation compared to sycamore maple. In addition, the present results show that competition in sycamore maple led to an increase in soluble protein concentration when grown with beech. This is consistent with previous studies (Simon et al., 2010b, 2014). Further experiments are required to test whether this common strategy is a consequence of *de novo* synthesis of proteins involved in the interaction between the two competing species. Soil N availability and the presence of a competing species also resulted in shifts in amino acid-N composition in the fine roots in both species. With high N supply, competition led to species-specific amino acid-N composition, whereas with intra-specific competition similar patterns were found suggesting that amino acid compositions shift depending on the competition regime even when N is available in excess. With N limitation, however, these patterns became less pronounced, because both had similar amino acid composition regardless of the competition regime indicating that soil N availability plays a major role in the competition for N between species.

Conclusion

Growth, N acquisition, and nutrition strategies of relatively slow-growing beech and relatively fast growing maple are adapted to N availability in the soil. The present results indicate that in beech-dominated forests on low N soil, the dominant tree species – beech – is optimally adapted to the environment. Intra- or inter-specific competition for N is avoided by different preferences for N sources in N acquisition. Furthermore, the understory provides the optimal environment to support beech seedlings rather than other woody plant species, such as sycamore maple which appears to be able to tolerate the conditions but at the cost of reduced growth and N acquisition capacity, therefore losing its competitive ability over beech. However, field studies are required to confirm these findings for the competition between beech and sycamore maple. Whether similar mechanisms of competition have been developed with other woody competitors, remains to be investigated.

Author Contributions

JS and XL conceived the experiment. XL conducted the experiment and analyzed the data. XL and JS wrote the paper. HR contributed ideas and improved the manuscript.

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Metabolomics differentiation of canola genotypes: toward an understanding of canola allelochemicals

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Allelopathy is one crop attribute that could be incorporated in an integrated weed management system as a supplement to synthetic herbicides. However, the underlying principles of crop allelopathy and secondary metabolite production are still poorly understood including in canola. In this study, an allelopathic bioassay and a metabolomic analysis were conducted to compare three non-allelopathic and three allelopathic canola genotypes. Results from the laboratory bioassay showed that there were significant differences among canola genotypes in their ability to inhibit root and shoot growth of the receiver annual ryegrass; impacts ranged from 14% (cv. Atr-409) to 76% (cv. Pak85388-502) and 0% (cv. Atr-409) to 45% (cv. Pak85388-502) inhibition respectively. The root length of canola also differed significantly between genotypes, there being a non-significant negative interaction ($r = -0.71$; $y = 0.303x + 21.33$) between the root length of donor canola and of receiver annual ryegrass. Variation in chemical composition was detected between organs (root extracts, shoot extracts) and root exudates and also between canola genotypes. Root extracts contained more secondary metabolites than shoot extracts while fewer compounds were recorded in the root exudates. Individual compound assessments identified a total of 14 secondary metabolites which were identified from the six tested genotypes. However, only Pak85388-502 and Av-opal exuded sinapyl alcohol, *p*-hydroxybenzoic acid and 3,5,6,7,8-pentahydroxy flavones in agar growth medium, suggesting that the synergistic effect of these compounds playing a role for canola allelopathy against annual ryegrass *in vitro*.

Keywords: *Brassica napus*, rapeseed, weed, root exudates, LC-QTOF-MS and metabolomics

INTRODUCTION

Weed control options for canola in Australia have been improved considerably with the development of a wide range of herbicide–tolerant cultivars with resistance to triazine, imidazolinone or glyphosate herbicides. The implementation of glyphosate-tolerant canola has changed the pattern of herbicide use, decreasing the use of other herbicides, and has given growers an efficient and simple solution for weed control worldwide (Harker et al., 2000; Beckie et al., 2011). Unfortunately, the use of herbicides in herbicide-tolerant canola cultivars has encouraged weeds to evolve herbicide-resistance (Powles et al., 1998; Heap, 2002). The ubiquitious weed annual ryegrass (*Lolium rigidum* L.) has already shown resistance to glyphosate in Australia (Pratley et al., 1999). Thus, herbicide resistance of weeds is a major threat to sustainable crop production. Consequently, alternatives to conventional synthetic herbicide application have become a focus of much research in Australia and worldwide. The potential use of crop allelopathy as part of a weed control program is one option gaining attention of the researchers (Kathiresan, 2005).

Rice (1984) defined allelopathy as the direct or indirect (harmful or beneficial) effect of a plant, and microbes, on another plant through the release of compounds into the environment. Allelochemicals have usually been considered to be secondary metabolites or waste products of the main metabolic pathways in plants (Swain, 1977) and released via several mechanisms (Seigler, 1996; Singh et al., 2003; Weston and Duke, 2003) including leaching (by dew and rain), residue decomposition (Putnam and DeFrank, 1983; Purvis et al., 1985) and exudation from living plants (Rice, 1984; Blum, 2011; Thorpe et al., 2011). Furthermore, the production and the release of biologically active compounds differ between species and between cultivars (Jeffery et al., 2003; Bennett et al., 2006; Keurentjes et al., 2006; Abdel-Farid et al., 2007), although relatively few have strong allelopathic properties (Bhowmik and Inderjit, 2003; Khanh et al., 2005; Xuan et al., 2005). The potential role of crop allelopathy in weed control has been the focus of much research and has been extensively reviewed (e.g., Einhellig and Leather, 1988; Purvis, 1990; Wu et al., 1999). Results from allelopathic assessment of canola cultivars against weeds *in vitro* and under field condition showed that canola allelopathy is genetically controlled (Asaduzzaman et al., 2014a,b). Canola allelopathy also seems to be independent from the competitive traits in the above ground morphology growth and phenology of the crop (Asaduzzaman et al., 2014c,d). However, there are no reports that holistically analyze the canola allelochemicals complex.

Plant secondary metabolites are generally present in plant tissue but few are exuded into the environment (Weston and Duke, 2003; Badri and Vivanco, 2009). To establish the involvement of any root exudates in crop plant allelopathy, it is important to demonstrate their phytotoxic effect by direct release to the growth medium (Inderjit, 1996). The exudation of allelochemicals by plant roots is an active metabolic process (Overland, 1966) and seems to be universal in the plant kingdom (Martin, 1957; Fay and Duke, 1977; Abdul-Rahman and Habib, 1989; Einhellig and Souza, 1992). Brassicaceae plants possess several groups of secondary metabolites including phenylpropanoids (hydroxycinnamates), flavonoids, as well as Brassicaceae-specific metabolites such as glucosinolates. The characterisation of these phytochemicals between strong and weak allelopathic cultivars is very important, as it will help to understand the chemical basis of canola allelopathy. Appropriate advanced tools, such as metabolomics, can be used for identifying and characterizing the potential metabolites responsible for the allelopathic defenses recently demonstrated in canola (Asaduzzaman et al., 2014a,b).

Metabolomics is an approach that allows a biochemical analysis of the total metabolite complement of a given plant tissue (Rinu et al., 2005; Kim et al., 2011). It is being used as an important procedure for identifying compounds involved in allelopathic interactions (D'Abrosca et al., 2013). Through mass spectral (MS) analysis of metabolomes in plant organs and principal component analysis (PCA), relative variability between organs can be explored. In addition, due to complex interactions, the field assessment of crop allelopathy is challenging (Inderjit and del Moral, 1997; Olofsdotter et al., 1999; Inderjit and Weston, 2000; Bertin et al., 2003; Bais et al., 2006) and difficult to separate from competition (Olofsdotter et al., 1999). Hence, laboratory screening of crop cultivars, coupled with advanced multivariate statistical analysis of metabolomes, offers new insights into the subterranean biology of plant allelopathy (Rinu et al., 2005).

The present research aimed to determine the metabolite composition of different organs (namely shoot, root) and root exudates of canola by using time-of-flight (TOF–MS) analysis technique and to establish a platform for understanding canola allelopathy.

MATERIALS AND METHODS

Plant materials

Six canola (*Brassica napus*, rapeseed, oilseed rape) genotypes were selected for this study namely: Av-opal, Pak85388-502, Av-garnet, Barossa, Cb-argyle and Atr-409. Previous field and *in vitro* screening results showed that Av-opal and Pak85388-502 were strongly allelopathic against annual ryegrass *in vitro,* and against the background weed populations (over 2 years: 2012 and 2013) under field conditions, whereas, Atr-409 and Barossa were weakly allelopathic genotypes (Asaduzzaman et al., 2014a,b). Two other genotypes were chosen based on a previous canola competitiveness field study conducted by Lemerle et al. (2014): Av-garnet was reported to be strongly competitive and Cb-argyle weakly competitive on weed species and associated total weed biomass. Seeds of these canola genotypes were obtained from the National *Brassica* Germplasm Improvement Program, located at NSW Department of Primary Industries, Wagga Wagga, NSW, Australia. Agar (technical grade) was purchased from Sigma Aldrich (St. Louis, MO, USA).

Sterilization and germination

Canola seeds were surface-sterilized by soaking in 2% sodium hypochlorite (NaOCl) for 5 min, then rinsed six times in sterilized distilled water. The seeds were transferred to a petri dish with one sheet of Whatman No. 1 filter paper, moistened with 5 ml sterilized distilled water, and sealed with parafilm. The surface-sterilized seeds of *Brassica* and ryegrass were kept in a 12-h light/12-h dark, 20/15°C controlled environment for 36 h and 48 h respectively.

General bioassay and growing conditions

The equal-compartment-agar-method (ECAM), described previously by Wu et al. (2000a) was chosen for bioassay. The method was developed based on the plant box method and relay seedling technique and separates competition and allelopathy phenomena between two simultaneously growing species. In this method, each species was placed into separate regions in the same container, where each species received equal space for its root system development. Briefly, glass beakers (600 mL, 12 cm depth, 8 cm diameter) containing 30 mL of 0.3% agar-medium (no nutrients, 1.3 cm depth) were autoclaved. The previous bioassay of 70 canola genotypes showed that 30 seedlings/beaker allelopathically gave greatest inhibition of the root length of annual ryegrass (Asaduzzaman et al., 2014a). Hence for each genotype, 30 uniform seedlings per beaker were chosen and aseptically transplanted from the germination dish onto one half of the agar surface, with the embryo up. The beaker tops were sealed with parafilm to prevent contamination and evaporation from the agar surface, and the beakers were placed in a controlled growth incubator with a daily 12-h light/12-h dark, 20/15°C cycle. Canola plants were grown for 6 days, 15 pre-germinated uniform seeds of annual ryegrass were aseptically sown on the other half of the agar surface at a distance of 4 cm from the canola seedlings. A piece of pre-autoclaved white paperboard was inserted across the center and down the middle of the beaker with the lower edge of the paperboard kept 1 cm above the agar surface. The beaker was divided into two equal compartments to minimize competition for space and light between the canola and ryegrass seedlings. The roots of canola freely entered the ryegrass compartment so that any allelochemicals produced and released by the canola seedlings can diffuse throughout the entire agar medium to influence ryegrass root growth. After ryegrass sowing, the beakers were again wrapped with parafilm and placed back in the growth chamber for 7 days. The receiver species, annual ryegrass, was also grown alone as a control. After 7 days, each annual ryegrass and canola seedling was carefully removed from the agar to avoid root breakage, and the root and the shoot lengths of 10 randomly selected plants within each beaker of both species were measured.

Experimental design and statistical analysis

A randomized complete block design was used for the experiment described. For each genotype 30 replicates were used in three different experimental units (beakers). The inhibited root and shoot length of annual ryegrass (mm) was converted as percentage of control. To determine the percentage change a percent ration was calculated between the mean root/shoot length of all ($n = 30$) ryegrass seedlings and the root/shoot length of every singly seedling. Further, to evaluate the equivalence of shoot and root inhibition of ryegrass with root length of canola, Pearson correlation co-efficient values were calculated. A linear regression analysis ($y = \mathrm{mx} + \mathrm{c}$) was also performed between root length (mm) of canola (independent) and of annual ryegrass (dependent) to know their mutual relationship. All data were subjected to analysis of variance using Genstat v13 (VSN International, Hemel Hempstead, UK) and the treatment means compared using the least significance difference (LSD) at a 5% level of probability. Plots of residual versus fitted values were examined for all traits to ensure the normality and homogenecity.

BIOCHEMICAL ANALYSIS BY METABOLOMICS APPROACH

Preparation of shoot and root extracts

Canola seedlings of each genotype were grown alone at a density of 30 seedlings/beaker for 13 days, as described in the above laboratory bioassay (see General bioassay and growing conditions). The roots and the shoots were cut from the canola seedlings and were immediately stored at −80°C in a sealed container. The frozen tissue was then freeze-dried for 24 h (Alpha 2–4 LD plus; John Morris). To extract metabolites, the freeze-dried tissue was then crushed to a fine powder using liquid nitrogen-chilled mortar and pestle. Sixty mg of the root and the shoot tissue of each canola genotype were placed separately into a 2 mL tube chilled in liquid nitrogen. The tube was filled with 400 μL 100% methanol solution containing internal standards $^{13}C_6$-sorbitol (0.5 mg/mL); $^{13}C_5{}^{15}N$-valine (0.5 mg/mL); penta-fluorobenzoic acid (0.25 mg/mL) and 2-aminoanthracene (0.25 mg/mL; Roessner and Dias, 2013). The tubes were vortexed for 30 s and centrifuged for 15 min at 13000 rpm at 4°C. The supernatant was transferred to a new pre-labeled 2 mL tube. An amount of 400 μL MQ water was added to the remaining pellet and vortexed, centrifuged and the supernatant was combined with the previous methanol containing supernatant. Three aliquots of each tissue containing 650 μL were prepared and stored at −80°C until analysis.

Collection of root exudates

Canola seedlings were carefully uprooted from their nutrient-free agar medium and the roots were rinsed twice with 5 mL portions of distilled water to remove any adhering agar and root exudates. The washings were pooled with the agar medium (30 mL). The agar medium was stirred carefully and extracted three times using 5 mL of 80% methanol. The extracted samples were vortexed and centrifuged and filtered through a 0.22 μm syringe filter into 2 mL labeled tubes. Three aliquots of 650 μL of each genotype were prepared and stored at −80°C before analysis.

Metabolites profiling by LC-QTOF-MS

To assess the metabolite composition differences among the organs and root exudates of canola genotypes, non-targeted and targeted metabolite profiling of extracted material was conducted. The compounds of canola shoots, roots extracts, and root exudates were separated on an Agilent 6520 LC-QTOF-MS system (Santa Clara, CA, USA, Agilent Mass Hunter Qualitative Analysis Build 6.0), with a dual sprayer ESI source, and attached to an Agilent 1200 series HPLC system (Santa Clara, CA, USA) consisting of a vacuum degasser, binary pump, with a thermo stated autosampler, column compartment, and diode array detector. The MS was operated in the negative mode using the following conditions: nebuliser pressure 45 psi, gas flow-rate 10 L/min, gas temperature 300°C, capillary voltage 3500 V, fragmentor 150 and skimmer 65 V. The instrument was operated in the extended dynamic range mode with data collected in mass-to-charge ratio (m/z), range 70–1700 amu.

Chromatography

An Agilent Zorbax Eclipse XDB-C18, 2.1 × 100 mm, 1.8 μm (Agilent) column was used with a flow rate of 400 μL/min maintained at ambient temperature (35 ± 1°C), resulting in operating pressures below 600 bar with a 12 min run time. A gradient LC-QTOF-MS method (**Table 1**) was used with mobile phases comprised of (A) 0.1% formic acid in de-ionized water and (B) 0.1% formic acid in acetonitrile. The sample run was conducted first for the 5 min by using linear gradient from 5% solvent (B) to 30% solvent (B), followed by a 5 min linear gradient to 30% solvent (B) to 100% solvent (B), then a 2 min hold at 100% solvent (B) and a 5 min re-equilibration at 5% solvent (B). Total time = 17 min. Three replications were run for each category of samples of each genotype.

Mass spectrum data processing

Relative qualitative analyses of the metabolites in the six canola genotypes were performed using Mass Hunter data analysis software (Agilent Technologies, USA). The extracted molecular features of each detected compound were matched with two different data bases (METLIN-AM-PCDL and HMDB-KEGG), plus the mass of the reference compounds from commercial standards. The individual compounds were also determined through assessing the outcomes of score (>70), hit count (total number of hits in the database) and mass differences (<5.0).

CHEMOASSAYS USING REFERENCE COMPOUNDS

Preparation of the different concentrations

Stock solutions (10000 μM) of sinapyl alcohol, *p*-hydroxybenzoic acid and 3,5,6,7, 8-pentahydroxy flavones were prepared separately. A mixture of these three compounds (10000 μM) was

Table 1 | Gradient of LC Method for 6520-QTOF.

Time (min)	A%	B%
0.00	95.0	5.0
5.00	70.0	30.0
10.00	0.0	100.0
12.00	0.0	100.0
12.10	95.0	5.0
17.00.	95.0	5.0

also made by using 1:1:1 ratio. The stock solutions of individual compounds and of their mixture were diluted to concentrations of 5000, 100, and 50 μM in HPLC-grade methanol.

Annual ryegrass bioassay with reference compounds

The modified chemical bioassay described by Seal et al. (2004b) was used to evaluate the phytotoxic effects of three reference compounds on annual ryegrass. One milliliter of each of the above concentrations (50, 100, 5000 and 10000 μM) was added to 600 ml beakers lined with Whatman #1 filter paper (Micro science, grade: MS 2 85 mm, size: 85 mm, Quality: 100) at the base. For the control, 1 ml of pure methanol was added. After the methanol had completely evaporated using the method described by Seal et al. (2004b), 5 ml of sterile double distilled water was added. Ten annual ryegrass seeds were sown directly into the water and the beaker was covered with parafilm. Three replicates of each treatment were arranged in a randomized complete block design in a growth chamber described in "General bioassay and growing conditions." 7 days later the annual ryegrass seedlings were removed from the system and both their root and shoot lengths were measured to the nearest 0.5 mm.

Statistical analysis

All dose-response curves were subjected to two-way ANOVA using Genstat v13 (VSN International, Hemel Hempstead, UK). Annual ryegrass root length (mm) was converted as percentage of control as described in "Experimental design and statistical analysis." The treatment means were compared using the LSD at a 5% level of probability. Plots of residual versus fitted values were examined for all traits to ensure that the assumptions of analysis of variance were met.

RESULTS

LABORATORY BIOASSAY

Genotypes differed significantly ($P < 0.001$) in their ability to suppress the root and the shoot growth of annual ryegrass (**Figure 1**). Genotypes Atr-409, Cb-argyl and Barossa showed less inhibitory effects on annual ryegrass while Av-opal, Pak85388-502 and Av-garnet were more inhibitive. In all collections, root growth (14–76%) of annual ryegrass was inhibited more than shoot growth (0–15%). The most suppressive genotype Pak85388-502 resulted in 76% root growth control of annual ryegrass followed by genotype Av-opal (74%) and Av-garnet (46%). The weakest genotype cv. Atr-409 inhibited the root length of annual ryegrass by only about 14%.

The average root length of canola seedlings differed significantly ($P < 0.001$) between genotypes (**Figure 2**). Genotypes Av-opal and Pak85388-502 produced the longest root; in contrast Cb-argyle and Atr-409 produced the shortest roots. The regression analysis ($r = -0.71$; $y = 0.303x + 21.33$) showed that annual ryegrass root growth (mm) was not decreased ($P > 0.05$) with increased canola root growth (mm).

METABOLITE PROFILING

The different metabolite patterns were observed by simple visual inspection of the MS traces of the three different organs. A total of 2806 mass signals were recorded in three different sample types.

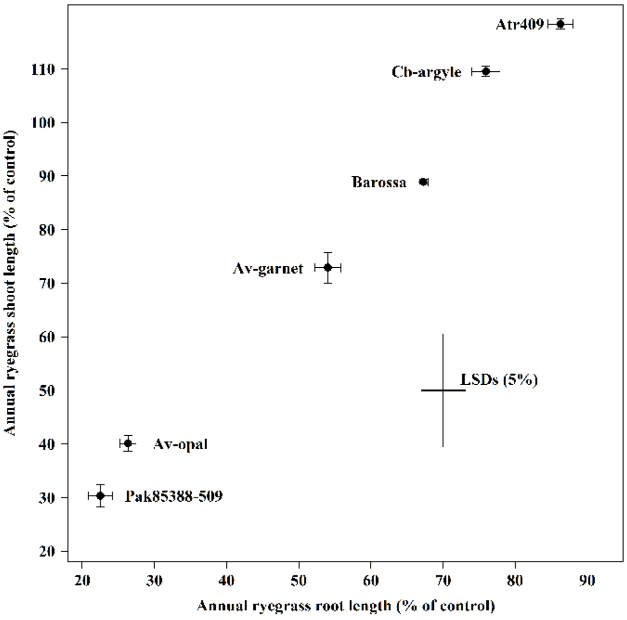

FIGURE 1 | Laboratory bioassays (ECAM) of canola (*Brassica napus*) seedling allelopathy against annual ryegrass (*Lolium rigidum*) seedlings. Data shown are the means ($n = 30$; ±SE) of the root and the shoot length (% of control) of annual ryegrass seedlings. Data were pooled of three experimental units (beakers). The cross indicates significant difference between the genotypes within two variables (root and shoot; $P < 0.05$, ANOVA with *post hoc* Fisher-LSD-test). The Pearson correlation coefficient (r) is 0.99 ($P < 0.001$).

The number of metabolites in the root and the shoot extracts varied between genotypes. Metabolites were highly enriched in root extracts followed by shoot extracts and root exudates (**Table 2**). Over 1807 compounds were found in roots, with Av-opal, Pak85388-502, Barossa and Atr-409 assigned 1586, 1532, 1471 and 1525 compounds respectively.

IDENTIFICATION OF PHYTOCHEMICALS IN CANOLA GENOTYPES

Fourteen secondary metabolites, including two internal signaling molecules, namely jasmonic acid and methyl-jasmonate, were detected across the samples of the six canola genotypes (**Table 3**). Only eight metabolites were identified in the root exudates.

The three interested metabolites were only found in the root exudates of highly allelopathic genotypes (Av-opal, Pak85388-502, and possibly Av-garnet). Five metabolites (or some mixture of these) were the most likely candidates for an allelopathic effect; sinapyl alcohol, *p*-hydroxybenzoic acid, quercitin, 3,5,6,7,8-pentahydroxy flavones, and methyl-jasmonate. Of these five, quercitin was formed only in the exudates of Av-garnet, and sinapyl alcohol was found only in the exudates of Av-opal and Pak85388-502.

CHEMOASSAYS USING REFERENCE COMPOUNDS

The root growth of annual ryegrass seedlings differed significantly ($P < 0.001$) between compounds and their concentrations

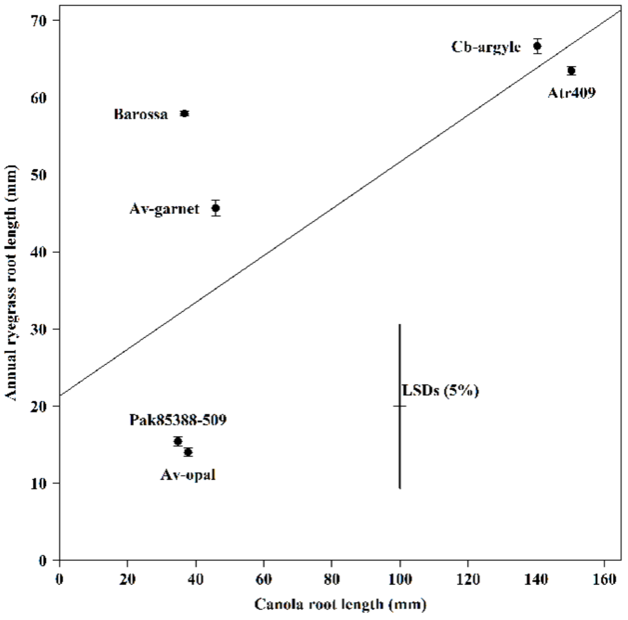

FIGURE 2 | The root length of canola (*Brassica napus*) and annual ryegrass (*Lolium rigidum*) seedlings when grown together in the ECAM bioassay. Data shown are means ($n = 30$;±SE) of the root length (mm) of annual ryegrass seedlings and of the root length (mm) of canola seedlings. Data were pooled of three experimental units (beakers). The cross indicates significant differences between the genotypes within one variable ($P < 0.05$, ANOVA with *post hoc* Fisher-LSD-test). The Pearson correlation coefficient (r) is −071 ($P > 0.05$) and were determined between the two variables across all genotypes and the regression equation is $y = 0.303x + 21.33$, with $R^2 = 0.50$, $P > 0.05$).

Table 2 | Total numbers of metabolites identified in root and shoot extracts and root exudates of six canola genotypes.

	Number of metabolites		
Genotype	**Root extracts**	**Shoot extracts**	**Root exudates**
Av-opal	1586	1494	908
Pak85388-502	1532	1496	951
Av-garnet	1436	1498	774
Barossa	1471	1402	920
Cb-argyle	1525	1524	888
Atr-409	1479	1479	957
Mean	1505	1480	899
LSD, $P < 0.001$	29	33	71

(**Figures 3** and **4**). Among the compounds 3,5,6,7,8- pentahydroxy flavones showed greater toxicity, while sinapyl alcohol was less toxic in all tested concentrations. When all tested compounds were considered together in mixture, the root growth of ryegrass was inhibited more compared to the individual effect of each compound, even in medium concentrations. Under the mixture of three compounds at, medium-to-high concentrations (100μM–10000μM) the germination ability of most of the ryegrass seeds was restricted.

DISCUSSION

Different inhibition activities against ryegrass seedlings were observed among the tested canola genotypes. This is in accordance with previous observations in rice (Seal et al., 2004a), wheat (Wu et al., 2000b), and rapeseed (Uremis et al., 2009), leading to the general conclusion that allelopathy is genetically controlled. The most allelopathic genotypes in this study were Av-opal and Pak85388-502, then competitive genotype Av-garnet. This suggests that root exudation from Av-opal and Pak85388-502 might also have played a significant role for its allelopathic activity in the bioassay. These two genotypes were previously characterized as highly allelopathic *in vitro* testing (Asaduzzaman et al., 2014a) and were also highly weed suppressive in the field (Asaduzzaman et al., 2014b).

The negative relationship between the root length of canola and annual ryegrass suggests that long roots of canola seedlings might produce more allelochemicals than short roots. Hence, despite vigorous shoot growth, Barossa and Av-garnet showed less root-exuded allelopathic activity, whereas the short vegetative growth but longer root growth of Av-opal still inhibited the root growth of annual ryegrass to a greater extent. Such findings also infer that the inhibition effects on the receiver plant were due to chemical interactions between the roots and that such chemicals were exuded into the agar by the canola roots. It seems possible that the allelopathy potential of any particular genotype depends upon firstly, the chemical composition of the root exudates, and secondly, the amount of chemical exuded which may be a function of root system length or surface area particular at later growth satge.

The biochemical analysis of canola organs and root exudates showed differences between genotypes in the production of their total metabolomes. It is to be expected that different canola genotypes will produce varying types and amounts of phytotoxic compounds since this has been shown to occur in various other crop species (Guenzi and McCalla, 1966; Fay and Duke, 1977; Wu et al., 2001; Jeffery et al., 2003; Fang et al., 2012; Farag et al., 2012). Gardiner et al. (1999) reported that the roots of rapeseed (*B. napus* L) contained more compounds than did the shoot. The root also contributed more to the total chemical pool for allelopathic activity (Gardiner et al., 1999). Similarly, in this study, the number of metabolites was generally higher in the root than in the shoot and in root exudates. Allelopathic research findings have also revealed that the allelochemical concentrations were higher in the roots than in the shoots of wheat (Wu et al., 2001). It is not clear whether the higher amounts of these allelochemicals in the roots result from their direct synthesis *in situ,* from their translocation from the shoots to the roots, or both. The presence of chemicals in the root exudates does not infer that they play any role in the observed phytotoxicity. However, it suggests that roots and shoots contain many compounds but only some are released as root exudates, depending upon particular conditions in the rhizosphere (Badri and Vivanco, 2009).

In previous *Brassica* allelopathy research, glucosinolates and their derivatives were proposed as potential allelochemicals of the crop's residue (Gardiner et al., 1999). These compounds were detected only in the root and the shoot extracts of three genotypes in this study. Possibilities for their non-detection in root exudates include: they remained locked inside the vacuole of fresh

Table 3 | Phytochemicals identified in shoot and root extracts and root exudates of six canola genotypes using LC-QTOF-MS in negative mode and matched with data from two data bases.

SL	Name	Formula	RT (min)	Mass	Score	m/z	Shoot extracts*	Root extracts*	Root exudates*
1	Malonic acid	$C_3 H_4 O_4$	0.696	104.011	73.64	104.01095	3, 4, 6	4, 6	4, 6
2	Isocitric Acid	$C_6 H_8 O_7$	0.931	192.0259	97.45	192.0210	1, 2, 3, 4, 5, 6	1, 2, 3, 4, 5, 6	1, 2, 3, 4, 5, 6
3	2-hydroxy-3,4-dimethoxybenzoic acid	$C_9 H_8 O_4$	4.857	180.043	76	180.04225	1, 2, 3, 4, 5, 6	1, 2, 3, 4, 5, 6	–
4	Sinapyl alcohol	$C_{11} H_{14} O_4$	4.987	210.087	94.06	210.08920	1, 2, 3, 4, 5, 6	1, 2, 3, 4, 5, 6	1, 2
5	Rutin	$C_{27} H_{3\ 0} O_{16}$	5.002	610.1559	78.19	610.15338	1, 2, 3, 4, 5, 6	1, 2, 3, 4, 5, 6	–
6	*p*-hydroxybenzoic acid	$C_7 H_6 O_3$	5.348	138.0303	78.37	138.03169	–	1, 2, 3, 4, 5, 6	1, 2
7	Vanillic acid	$C_8 H_8 O_4$	5.59	168.0414	81.29	168.04225	1, 2, 3, 4, 5, 6	1, 2, 3, 4, 5, 6	–
8	trans-3-hydroxycinnamic acid	$C_9 H_8 O_3$	6.356	164.0458	73.8	164.0473	1, 2, 3, 4, 5, 6	–	–
9	Dimethoxy-4-hydroxycinnamic acid	$C_{11} H_{12} O_5$	6.631	224.0693	98.34	224.06847	–	1, 2, 3, 4, 5, 6	1, 2, 4, 6
10	2-phenylethyl glucosinolates	C_9H_9NS	6.832	163.24	90.03	163.04556	2, 4,5	2, 4, 5	–
11	Quercitin	$C_{15} H_{10} O_7$	7.159	302.046	69.87	302.04265	1, 2, 3, 4, 5, 6	1, 2, 3, 4, 5, 6	3
12	3,5,6,7,8 pentahydroxy flavone	$C_{15}H_{10} O_7$	7.50	302.205	70.05	302.04265	–	1,2	1, 2
13	Jasmonic acid	$C_{12} H_{18} O_3$	8.224	210.1224	81.95	210.12559	–	1, 2, 3, 5	–
14	Methyl jasmonate	$C_{13} H_{20} O_3$	9.541	224.1386	72.05	224.14124	–	1, 2	1, 2

**Number indicates whether the compound is found in the tissue of the six genotypes: 1, Av-opal; 2, Pak85388-502; 3, Av-garnet; 4, Barossa; 5, Cb-argyle; 6, Atr-409; "–," not present.*

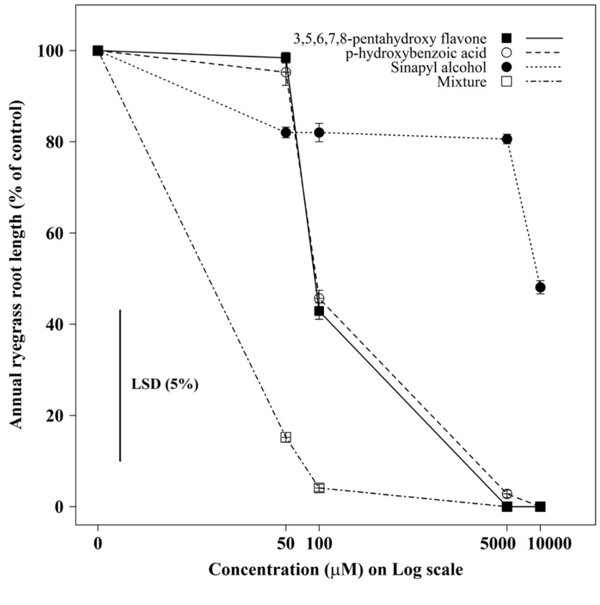

FIGURE 3 | The individual, and combined, effect of sinapyl alcohol, *p*-hydroxybenzoic acid, and 3,5,6,7,8-pentahydroxy flavone on the root growth of annual ryegrass seedlings. Data shown are the means ($n = 30$; ±SE) of the root length of annual ryegrass seedlings. Data were pooled of three experimental units (beakers). The cross indicates significant difference between the genotypes × concentration interaction within one variables ($P < 0.05$, ANOVA with *post hoc* Fisher-LSD-test).

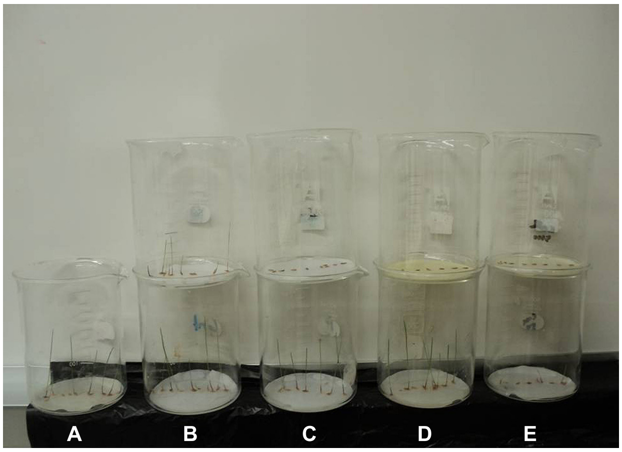

FIGURE 4 | Comparison of the seedlings growth of annual ryegrass affected by (A) control, (B) sinapyl alcohol, (C) *p*-hydroxybenzoic acid, (D) 3,5,6,7,8-pentahydroxy flavone and (E) their mixture. The beakers in the bottom were treated with low concentration (50 μM), while beakers on the top were treated with high concentration (10000 μM).

tissue of living plant; or they could not be detected due to their complex volatile nature. Glucosinolates were not detected in the root exudates from living tissue of any genotypes showing high allelopathy in our study. Therefore it seems unlikely that they are responsible for allelopathy. This conclusion is most striking when comparing the consistent results from the three replications of the tested genotypes, including Av-opal and Pak85388-502. Both are highly allelopathic but Av-opal is low in glucosinolates in the seed while Pak85388-502 is high in glucosinolates in the seed (Asaduzzaman et al., 2014b). Glucosinolates and their breakdown products are significant in the phytotoxic effects observed for canola stubble and stubble leachates after harvest (Boydston and Hang, 1995; Brown and Morra, 1996; Al-Khatib et al., 1997). It may be that senescence (aging) and fallen leaves may make a contribution to weed suppression during the life cycle of the crop but this has not been specially recorded. The cut and green manure rapeseed suppressed weeds (Boydston and Hang, 1995) but this may be due to physical smothering rather than chemical effects.

Several potential allelopathic compounds were found in the root and the shoot tissue in this study but were not detected in root exudates. This suggests that the expression of the allelopathic effect not only depends on particular compounds being synthesized but also on the ability of the genotypes to actively exude these into their rhizosphere. For instance, Barossa and Atr-409, the two weakly allelopathic genotypes, contained potential phytotoxic metabolites in the roots and the shoots but their inhibitory effect on annual ryegrass was weak. Dicarboxylic malonic acid was found only in the root exudates of these two weakly allelopathic genotypes and this compound may act as a buffering agent to reduce the threshold levels of other potential allelochemicals in the rhizosphere. Similar results have been also reported in rice (Seal et al., 2004b), where the amounts of dicarboxylic acids was high in root exudates of non-allelopathic rice cultivars.

Sinapyl alcohol, *p*-hydroxybenzoic acid and 3,5,6,7,8-pentahydroxy flavone were isolated from root exudates of the two strongly allelopathic canola genotypes, suggesting that they were at least partly responsible for the observed allelopathic activity. The detection of two signal molecules (jasmonic acid and methyl-jasmonate) in the allelopathic genotypes also supports the proposition that they are also involved in canola allelopathy. Jasmonic acid and methyl-jasmonate act as secondary messengers in signal transduction events in the cell and have inhibitory effects on many plant physiological processes (Sembdner and Parthier, 1993). Abdel-Farid et al. (2007) reported that the accumulation of these signal molecules is connected with demand or synthesis of the secondary metabolites sinapyl alcohol and *p*-hydroxy benzoic acid in *Brassica rapa*. Furthermore, 3,5,6,7,8- pentahydroxyflavone was also detected previously in root exudates of another member of the Brassicaceae, *Brassica alba* (Ponce et al., 2004). *p*-hydroxybenzoic acid has been reported as a potential allelochemical in other crops including, *Glycine max* (Barkosky and Einhellig, 1993), *Camelina alyssum* (Grummer and Beyer, 1960), and several members of the genus *Althaea* (Gude and Bieganowski, 1990). Some of the reduction in root and coleoptile growth of wheat seedlings caused by wild oat (*Avena fatua*) root exudates is attributed to this compound (Perez and Ormeno-Nunez, 1991).

It has been postulated that allelopathic effects are most likely due to the combination and interaction of a complex mixture of compounds (Rizvi and Rizvi, 1992; An et al., 2001). The chemobioassay results of the present study revealed that, the allelopathic activity of canola cultivars resulted from the synergistic effects of sinapyl alcohol, *p*-hydroxybenzoic acid and 3,5,6,7,8-pentahydroxy flavones. It is possible that multiple compounds present at low concentrations can have pronounced allelopathic effects through their joint action, though evidence for this elusive. Joint allelopathic interactions between compounds have also been reported in several tested species including rice (Chou et al., 1991; Seal et al., 2004b) and vulpia (An et al., 2001).

The phytotoxicity observed among the tested canola genotypes indicates that allelopathy plays a role in inhibiting the annual ryegrass weed species. Field experiments (Asaduzzaman et al., 2014b) support this conclusion. The comprehensive chemical analysis reported here revealed that sinapyl alcohol, *p*-hydroxybenzoic acid and 3,5,6,7,8-pentahydroxy flavones in most suppressive genotypes (cv. Av-opal and Pak85388-502) are likely allelopathic agents via root exudates in canola.

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Resource competition in plant invasions: emerging patterns and research needs

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Invasions by alien plants provide a unique opportunity to examine competitive interactions among plants. While resource competition has long been regarded as a major mechanism responsible for successful invasions, given a well-known capacity for many invaders to become dominant and reduce plant diversity in the invaded communities, few studies have measured resource competition directly or have assessed its importance relative to that of other mechanisms, at different stages of an invasion process. Here, we review evidence comparing the competitive ability of invasive species vs. that of co-occurring native plants, along a range of environmental gradients, showing that many invasive species have a superior competitive ability over native species, although invasive congeners are not necessarily competitively superior over native congeners, nor are alien dominants are better competitors than native dominants. We discuss how the outcomes of competition depend on a number of factors, such as the heterogeneous distribution of resources, the stage of the invasion process, as well as phenotypic plasticity and evolutionary adaptation, which may result in increased or decreased competitive ability in both invasive and native species. Competitive advantages of invasive species over natives are often transient and only important at the early stages of an invasion process. It remains unclear how important resource competition is relative to other mechanisms (competition avoidance via phenological differences, niche differentiation in space associated with phylogenetic distance, recruitment and dispersal limitation, indirect competition, and allelopathy). Finally, we identify the conceptual and methodological issues characterizing competition studies in plant invasions, and we discuss future research needs, including examination of resource competition dynamics and the impact of global environmental change on competitive interactions between invasive and native species.

Keywords: evolutionary adaptation, apparent competition, dominance, competitive ability, phenology, phenotypic plasticity, phylogenetic relatedness, resource gradient

INTRODUCTION

Invasions by alien plants represent a major component of global change (Vitousek et al., 1996). Successful invasions occur when an alien species is capable of forming self-sustaining populations (naturalization) that may extend at considerable distances away from the original source of introduction, overcoming a range of biotic and abiotic barriers, along an introduction-naturalization-invasion continuum (Richardson et al., 2000; Richardson and Pyšek, 2012). Invasive alien species are known to alter the composition and diversity of the aboveground (e.g., Levine et al., 2003; Gaertner et al., 2009; Hejda et al., 2009) and belowground flora (Gioria and Osborne, 2010; Gioria et al., 2014) of many recipient communities, as well as impacting on a range of abiotic and biotic conditions, with potentially significant changes in the functioning of, and the services provided by, invaded ecosystems (see Ehrenfeld, 2010; Simberloff, 2011; Vilà et al., 2011; Eviner et al., 2012; Pyšek et al., 2012).

Resource competition, also known as exploitative competition, is a key process regulating plant community dynamics (e.g., Grime, 1973, 1977; Newman, 1973; Harper, 1977; Tilman, 1982, 1988) and has long been considered as a major mechanism determining the success of several invasive species (Elton, 1958; Tilman, 1997; Levine et al., 2003; Vilà and Weiner, 2004). In particular, a capacity for many invasive species to reduce diversity and to form nearly mono-specific stands (e.g., Beerling et al., 1994; Tiley et al., 1996; Gaertner et al., 2009) has often been attributed to a superior capacity of invasive species to compete for resources (Levine et al., 2003; Vilà et al., 2003; Vilà and Weiner, 2004) and/or due to the disproportionately greater effects of increases in resources on the performance of invasive vs. native species (e.g., Daehler, 2003; Leishman and Thomson, 2005; Funk, 2013).

In this paper, we review the literature on the role of resource competition in plant invasions. Specifically, we review studies comparing the competitive ability of invasive species vs. that of co-occurring native species, along a range of environmental gradients, distinguishing between the importance of competitive interactions below- and above-ground as well as that of intra- vs. interspecific competition; we report on the findings of studies accounting for phylogenetic relatedness through comparisons of invasive and non-invasive congeners, and of those comparing the competitive ability of dominant aliens vs. dominant natives; we discuss how the outcomes of resource competition may depend on other mechanisms, including phenotypic plasticity as well as evolutionary adaptation that may lead to increased or decreased competitive ability in both invasive and native species, dispersal and recruitment limitation, and competition avoidance that may result from phenological differences or from niche differentiation in space; we report on how indirect competition and allelopathy may interact with resource competition and on how a superior competitive ability of invasive species over that of native species may be only transient and change over time. Finally, we identify the conceptual and methodological issues characterizing research needs in this field and discuss future research directions, including examinations of the potential impact of global environmental changes on resource competition between invasive and native species.

COMPETITIVE ABILITY IN INVASIVE SPECIES

DEFINING RESOURCE COMPETITION, COMPETITIVE ABILITY AND DOMINANCE

Two major questions in the field of invasion ecology relate to the competitive ability of invasive species and how this is affected by resource availability: (1) are invasive species superior competitors over native species; and (2) how do competitive interactions for resources between invasive and native species vary (over time) along environmental gradients? (see Glossary).

Resource competition is a negative interaction between individuals associated with a requirement for shared limiting resources (light, nutrients, and water) resulting in a reduction in one or more fitness components at the individual or at the population level (see Glossary; Goldberg et al., 1999). From a functional point of view, competition can be regarded as an alteration of the processes of (1) "acquisition" of resources, (2) their "allocation" to different parts, and contribution to overall plant performance and (3) the "deployment" of these parts in space (Bazzaz, 1996), by neighboring individuals.

Based on the above definitions, the competitive ability of a species can be broadly regarded as the ability of a species to acquire and/or make the best use of limiting resources, and/or a capacity to cope with low resource levels or to reduce the availability of resources to its neighbors. Such an ability is dependent on a combination of species traits that allow a species to compete for resources with neighboring individuals or species (see Weiner, 1993), including relative growth rate, height, lateral spread, storage organs, shoot thrust, leaf and root longevity, leaf nutrient concentration, specific leaf area, active foraging capability, response to damage, and palatability (Grime, 1998). Individual plants, however, vary greatly in their tolerance to different levels of available resources, making the concept of competitive ability at the species level strongly context-dependent (Tilman, 1982, 1988; see Weiner, 1993).

Goldberg (1990) pointed out that the competitive ability of a species can be classified into two components, as each individual has an effect on and responds to its environment, including its neighbors: (1) a "competitive effect", which can be regarded as the ability of an individual to take up resources (high rates of resource acquisition), ultimately resulting in a reduction in the resources available to neighboring plants; and (2) a "competitive response," i.e., the response of a species to reduced resource levels by competitors, which depends on a species' ability to tolerate low resource conditions associated with the presence of neighbors. These mechanisms, which can also be summarized into maximum resource capture vs. minimum resource requirements are not mutually exclusive (Suding et al., 2004).

Invasive species may achieve dominance via an innately superior competitive ability over that of native species arising from physiological advantages that include high rates of resource acquisition (e.g., Eliason and Allen, 1997; Alpert et al., 2000; Callaway and Aschehoug, 2000; Rejmánek, 2000; Pyšek and Richardson, 2007; van Kleunen et al., 2010, 2011; Matzek, 2012; Funk, 2013), such as a capacity to fix nitrogen (e.g., Atwood et al., 2010; Le Maitre et al., 2011; Gioria and Osborne, 2013) or an ability to tolerate low resource availability (see Tilman, 1982; Weiner, 1993; Goldberg, 1996; Craine et al., 2005; Funk, 2013). Dominance can also be achieved via mechanisms that may interact with resource competition and that will be discussed throughout this paper, including: (1) competitive advantages arising from the release from natural enemies that are present in their native range but not in the invasive range (Enemy Release Hypothesis; ERH; Keane and Crawley, 2002; Mitchell and Power, 2003; Callaway et al., 2004; Maron et al., 2014); (2) an increased competitive ability post-introduction arising from evolutionary changes leading to a reallocation of resources from defense mechanisms that may be required in their native range, to growth and development (Evolution of Increased Competitive Ability; EICA; Blossey and Nötzold, 1995); (3) high phenotypic plasticity in traits that allow the survival and spread in areas characterized by novel conditions (Bossdorf et al., 2005; Richardson and Pyšek, 2006; Davidson et al., 2011); (4) trait differences between alien and native species arising from phylogenetic distinctiveness (Mack, 1996; Rejmánek, 1996) and potentially reflecting differences in the ecological niches that can be occupied. This is based on early observations suggesting that competition with native species would favor the establishment of taxonomic distinct alien species (Darwin's Naturalization Hypothesis; Darwin, 1859); (5) pre-existing or acquired (via phenotypic and/or evolutionary responses) phenological differences that allow alien species that emerge earlier or persist longer to avoid resource competition in the early/later stages of development (Weiner, 1993; Wolkovich et al., 2013); (6) an ability to alter the abiotic (Ehrenfeld, 2010; Vilà et al., 2011; Pyšek et al., 2012) and biotic conditions in the invaded communities (White et al., 2006; Kulmatiski et al., 2008); (7) the release of allelochemicals that are potentially toxic to native species (Callaway and Aschehoug, 2000).

While the ERH predicts that the release from enemies confer an immediate competitive advantage to alien species, according to EICA, the invasive potential of alien species depends, at least in part, on their ability to evolve to reallocate resources previously destined to defense from natural enemies, thus they do not need to be competitively superior over native species at the time of introduction into a community. Evidence for ERH, EICA, and the Darwin's Naturalization Hypothesis is strongly context-dependent (e.g., Daehler, 2001; Duncan and Williams, 2002; van Kleunen and Schmid, 2003; Hierro et al., 2004; Bossdorf et al., 2005; Schaefer et al., 2011).

ARE INVASIVE SPECIES SUPERIOR COMPETITORS OVER NATIVE SPECIES?

Resource competition may play an important role in both the establishment (naturalization phase) and the spread (invasion phase) of invasive alien species, as well as in determining the magnitude and direction of the impact of plant invasions on invaded communities. Competitive advantages associated with a superior capacity to acquire resources have been regarded as a key factor responsible for the dominance of many alien species (*sensu* Grime, 1998) in the invaded communities (e.g., Tilman, 1997; Levine, 2000; Shea and Chesson, 2002; Levine et al., 2003; Seabloom et al., 2003; Stachowicz and Tilman, 2005).

Whether or not invasive plants are competitively superior over co-occurring native species thus represents a central question in invasion ecology. The competitive ability of invasive species has been compared to that of native species in several experimental studies in which co-occurring invasive and native species were grown separately or in mixtures, along a range of environmental conditions (see Vilà and Weiner, 2004; White et al., 2006 for reviews). Several studies have shown that many invasive species possess higher values of competitively advantageous traits than native and non-invasive species, including a superior capacity to acquire and retain resources and/or to advantageously exploit resources better than co-occurring native species (e.g., Huenneke et al., 1990; Burke and Grime, 1996; Rejmánek, 1996; Callaway and Aschehoug, 2000; Daehler, 2003; Leishman and Thomson, 2005; van Kleunen et al., 2010; Matzek, 2012), even in ecosystems with low-resource availability (Tecco et al., 2010; Funk, 2013). Also, there is evidence that resource competition does not necessarily play an important role in determining dominance by invasive plants (e.g., Mangla et al., 2011a) and a superior competitive ability is not a necessary condition for successful invasions (e.g., Corbin and D'Antonio, 2004; McGlone et al., 2012).

Conceptual and methodological issues have characterized many competition studies in invasion ecology, potentially affecting our understanding of the role of resource competition in plant invasions. First, the majority of studies have characterized the competitive ability of co-occurring invasive and native species indirectly, mainly by testing differences in biomass or other measures of plant growth or fitness, thus focusing on the *outcomes* of competition and between invasive and native species, rather than the *process* of competition (see Trinder et al., 2013 for a discussion between direct and indirect measurements of plant competition). Another potentially significant issue is associated with the fact that the majority of studies on resource competition between invasive and native species have focused on measuring biomass at one point in time or on final harvest data, and have not accounted for the dynamic nature of this process (see Trinder et al., 2013).

A potential source of bias in the interpretation of competition studies in invasion ecology is associated with the fact that several experiments have compared the competitive ability of invasive (dominant alien species) vs. that of native subordinate or transient species (*sensu* Grime, 1998). In this case, the selected native species would likely be negatively affected by resource competition with any dominant species, regardless of its native/alien status. This bias was evident in Vilà and Weiner's (2004) review of pair-wise competition experiments, which supported the general notion that invasive species are good competitors, although the authors warned that dominance by invasive species could depend on effects other than those associated with resource competition, including indirect competition, allelopathy (see Glossary; Weidenhamer et al., 1989; White et al., 2006), or phylogenetic and life form differences between species pairs.

The competitive ability of invasive vs. native species is dependent on the environmental conditions encountered in the introduced range (e.g., Alpert et al., 2000). Several authors have pointed out that evidence for a superior competitive ability of invasive species might be biased by the fact that the majority of studies have been conducted in highly productive environments (e.g., Kueffer et al., 2007), where invasive species tend to be better competitors than native species via a superior capacity to acquire resources more effectively than native species (e.g., Daehler, 2003; Matzek, 2012).

Finally, few studies have assessed the importance of resource competition relative to that of other mechanisms (e.g., Levine et al., 2003; Vilà and Weiner, 2004; White et al., 2006). In a review of 150 papers examining the impacts of alien plants, Levine et al. (2003) showed that fewer than 5% of those studies had confirmed the mechanisms responsible for the impact of alien plants (competition, allelopathy, or other processes), despite the majority having identified resource competition as a major mechanism underpinning their findings. The following provides is information on what is known about the competitive ability of invasive species vs. that of native species across a range of resource gradients, and on the factors that may hinder our capacity to assess the importance of resource competition in plant invasions.

COMPETITION FOR NUTRIENTS

Variations in the competitive ability of invasive species along resource gradients have received considerable attention (e.g., Grime, 1973, 1977, 2001; Newman, 1973; Tilman, 1982, 1988, 1997; Davis et al., 1998, 2000; Suding et al., 2004; Gross et al., 2005). A superior ability to acquire nutrients has been regarded as a major determinant of the successful establishment, spread, and persistence of invasive species, particularly in highly productive environments (e.g., Burke and Grime, 1996; Matzek, 2012), and several studies have shown that nutrient enrichment can be disproportionately more beneficial to invasive species than to natives (e.g., Huenneke et al., 1990; Witkowski, 1991; Milchunas and Lauenroth, 1995; Burke and Grime, 1996; Daehler, 2003; Lowe et al., 2003; Leishman and Thomson, 2005; Vinton and Goergen, 2006; Abraham et al., 2009; Sharma et al., 2010). Moreover, temporary increases in nutrient levels associated with natural or anthropogenic disturbances (see Glossary) may mitigate the negative effects of competition for nutrients (e.g., reduction in growth or lateral spread) with native species (Quinn et al., 2007), although the intensity of competition for nutrients may increase with increases in N availability (Mangla et al., 2011b). In contrast, decreases in nutrient levels may reverse the outcome of competition, with native species performing better than invasive species under low nutrient levels (e.g., Wedin and Tilman, 1993; Claassen and Marler, 1998).

Despite being regarded as better competitors for nutrients compared to native species in productive environments, many invasive species have also colonized unproductive environments (Groves et al., 2003; Funk, 2013). Increases in nutrient concentrations associated with natural or anthropogenic disturbances may promote plant invasions in these environments via a disproportionately beneficial effect on the competitive ability of invasive species over that of native ones, such as in serpentine ecosystems (O'Dell and Claassen, 2006) or in coastal dune communities (French, 2012).

In addition to possessing a superior capacity to acquire nutrients, many invasive species are known to reduce the level of nutrients available to co-occurring native species in invaded communities. For instance, Callaway and Aschehoug (2000) showed that the Eurasian forb, *Centaurea diffusa*, which is invasive in North America, had negative effects on nutrient (^{32}P) uptake in North American bunchgrass species, likely due to a differential ability to use nutrients compared to native species. Suding et al. (2004) also showed that *C. diffusa* is better able to use P and is less limited by N compared to co-occurring native species in invaded communities, while, under low P, it appears to lose its competitive advantage and its response to resource competition is similar to that of native species.

COMPETITION FOR WATER

A superior capacity to compete for water may play a major role in promoting the establishment of alien species (e.g., Thebaud et al., 1996). In environments characterized by low water availability, native species are expected to be better competitors for water than alien species, due to a presumed adaptation to periodic water deficits. However, there is evidence showing that invasive species are better competitors even in environments characterized by low water availability (Nernberg and Dale, 1997; López-Rosas and Moreno-Casasola, 2012; Mason et al., 2012). For instance, Nernberg and Dale (1997) showed that the competitive ability of five native grasses was lower than that of the alien grass *Bromus inermis*, even under water stress. Mason et al. (2012) tested competition effects along gradients of water availability for a representative suite of species from coastal dune communities that had been invaded by *Chrysanthemoides monilifera* subsp. *rotundata* and showed that native species were often weak competitors compared to the invader, even under water stress, despite their adaptation to periodic water stress in native coastal environments, although native shrub species that are functionally similar to the invader were more effective at competing against the invader.

COMPETITION FOR LIGHT

Competition for light is generally regarded as an asymmetric type of competition (Yokozawa and Hara, 1992), which occurs when larger individuals obtain a disproportionate share of resources, relative to their initial size, suppressing the growth of smaller individuals (Begon, 1984; Weiner and Thomas, 1986; Keddy and Shipley, 1989; Weiner, 1990; Gerry and Wilson, 1995; Connolly and Wayne, 1996; Freckleton and Watkinson, 2001; see Glossary). Competition for light is considered a major determinant of the successful establishment of alien species, with many invaders outcompeting native species via a superior ability to capture light and via subsequent shading effects associated with a higher biomass production compared to natives (e.g., Hobbs and Mooney, 1986; Maule et al., 1995; Hutchinson and Vankat, 1997; Richardson et al., 2000; Morris et al., 2002; Kueffer et al., 2007; Iponga et al., 2008) and/or through related traits, such as a higher specific leaf area (e.g., Smith and Knapp, 2001; Iponga et al., 2008). For instance, Morris et al. (2002) showed that improved light capture and a greater stem elongation rate conferred the invasive shrub *Ligustrum sinense* with a competitive advantage over the native shrub *Forestiera ligustrina*. Such a superior competitive ability in light acquisition appeared to explain its higher photosynthetic capacity and resource use efficiency, as well as the observed fruit production of the invasive *L. sinense* vs. *F. ligustrina* (Morris et al., 2002). A superior capacity to compete for light compared to native dominant trees (*Acacia tortilis* and *Rhus lancea*) was reported to promote invasions by the alien tree *Schinus molle* in semi-arid savannas in South Africa (Iponga et al., 2008). Such a superior competitive ability was observed in alterations in canopy symmetry in native trees that were associated with a degree of intolerance to shading caused by the invader.

The formation of a large biomass by many invasive species is often associated with their superior capacity to compete for light and contributes to determining the magnitude of the impact of invasive species on native communities via shading effects (Grime, 2001). However, an invader's large canopy and/or biomass may be due to a superior capacity to compete belowground for nutrients and water (e.g., Coomes and Grubb, 2000; Kueffer et al., 2007) rather than to a superior capacity to compete for light. Moreover, leaf dynamics or architecture may be more important than a large shoot biomass *per se* in conferring a high competitive ability (e.g., Grime, 2001). Assessments of competition for light should thus be examined in combination with assessments of the effects of competition for belowground resources.

COMPETITION FOR SPACE

The allocation to vegetative vs. reproductive tissues is a function of the availability of underground space (McConnaughay and Bazzaz, 1991). Despite the fact that physical space is not a consumable resource (McConnaughay and Bazzaz, 1991; Bazzaz, 1996), its effects on the access to other resources such as water, nutrients, and light could play an important role in determining the outcomes of resource competition between alien and native species. The majority of studies referring to space constraints have examined patterns of invasions following disturbances creating gaps (increases in light availability) that can be colonized by ruderal invaders (e.g., D'Antonio and Vitousek, 1992; Hobbs and Huenneke, 1992; Thompson et al., 2001; Buckley et al., 2007), while the effects of space on competitive interactions between invasive and native species represent a major research need (Gao et al., 2014) that requires further investigation.

THE IMPORTANCE OF PHENOLOGY

Phenological differences resulting in early growth and in the initiation of significant size increases prior to those of native species may have a large impact on competitive interactions between invasive and native species (e.g., Tiley et al., 1996; Caffrey, 2001; Standish et al., 2001; Sala et al., 2007; Gioria and Osborne, 2010, 2013; Wilsey et al., 2011; Wolkovich and Cleland, 2011; Funk, 2013; Wolkovich et al., 2013). Early growth allows a species to exploit the available resources before other species and avoid competition for some resources during the initial stages of plant development. Thus, in the presence of phenological differences that allow an invasive species to grow earlier than native species, a high competitive ability in the invader may be less important or made unnecessary.

Drought avoidance is one particular example. In arid and semi-arid ecosystems, invasive species are not necessarily less drought-tolerant than native species (e.g., Williams and Black, 1994; Cleverly et al., 1997; Nernberg and Dale, 1997), and the successful establishment of some annual invaders is associated with a capacity to avoid drought stress (e.g., Solbrig, 1986; Fox, 1992) by completing their life cycles over the short period when water availability is high (see Alpert et al., 2000 and references therein). In some cases invasive species may possess a combination of water deficit evasion and tolerance mechanisms (Baruch and Fernandez, 1993). In a review of species traits of invasive species in low-resource environments, Funk (2013) showed that, in arid and semi-arid environments, three studies out of three showed that early germination was more pronounced in invasive rather than in native species under low water availability, indicative of potential phenotypic and/or adaptive responses to low water availability of invasive species resulting in phenological changes.

Phenological differences between invasive and native species represent a major confounding factor in determining the role of resource competition in the successful establishment of early growing alien species. Such differences should be accounted for as they allow an invasive species to avoid resource competition during the initial phase of development and confer an invader with competitive advantages (resource pre-emption) that are due to a capacity for early growth rather than to a superior competitive ability.

INTER- AND INTRA-SPECIFIC COMPETITION

Competition at the early stages of plant development associated with small differences in initial size and growth between neighboring individuals may have long-term effects on competitive interactions (Weiner, 1993; Foster and Gross, 1997, 1998; Suding and Goldberg, 1999; Mangla et al., 2011b). Both interspecific competition between invasive and native species and intraspecific competition may thus affect the competitive ability of invasive and native species. To date, few studies have, however, examined the role of intra- and interspecific competition in determining the outcomes of competitive interactions between invasive and native species, and the results appear to be strongly species-specific.

For native species, interspecific competition with alien species appears to be the predominant form of competition (Lowe et al., 2003; Vasquez et al., 2008; Young and Mangold, 2008; Mangla et al., 2011b), although intraspecific competition may be important in determining the initial size of native seedlings, with potential effects on the outcome of competition with invasive seedlings (Mangla et al., 2011b). For some invasive species, intraspecific competition is often the predominant type of competition (Lowe et al., 2003; Vasquez et al., 2008; Young and Mangold, 2008; Blank, 2010; Mangla et al., 2011b; Skálová et al., 2013), likely reflecting stronger differences in competitive ability between invasive and native species than among individuals of the same species. For instance, examination of inter- and intraspecific competition among four native and invasive *Impatiens* species, Skálová et al. (2013) found that the invasive *I. parviflora* competed better in intra- vs. interspecific competition, while the invasive *I. glandulifera* performed better under interspecific competition with its congeners, although it may form a large aboveground biomass even in intraspecific competition experiments (Bottollier-Curtet et al., 2013).

The importance or intensity of intra- vs. interspecific competition may differ with the stage of the life cycle (e.g., Young and Mangold, 2008; Mangla et al., 2011b), since individual plants go through various physiological stages as they develop and competition occurs within and between stages for different individuals (Connell, 1983; Cameron et al., 2007; Mangla et al., 2011b). For instance, Mangla et al. (2011b) performed a range of competition experiments that tested the intensity and importance of intra- and inter-specific competition between two invasive annual grasses (*Bromus tectorum* and *Taeniatherum caput-medusae*), which are native to Eurasia and the Mediterranean region, and two native perennial grasses (*Pseudoroegneria spicata* and *Poa secunda*) that co-occur in their invasive range. They showed that native perennial grasses were subject to both intra- and interspecific competition with invasive annual species during early growth stages, but the type of competition differed among four harvests. This suggests that the relative importance of intra- vs. interspecific competition varies among harvests during the early stages of plant growth (Mangla et al., 2011b; see Trinder et al., 2013) and emphasizes the importance of examining competition at several points in time (Foster and Gross, 1997, 1998; Gibson et al., 1999), particularly when comparing species characterized by different life cycles (Gibson et al., 1999).

Bossdorf et al. (2004) warned that experiments aimed at identifying potential mechanisms leading to the successful establishment of invasive species may provide contrasting outcomes depending on whether the effects of intraspecific competition are accounted for or not, given that, under intense intraspecific competition, invasive populations may have lower fitness (van Kleunen and Schmid, 2003) and a reduced competitive ability (Bossdorf et al., 2004). Future studies should address this research gap, given the importance that intraspecific competition may play, particularly at the initial stages of invasion.

ABOVE- AND BELOW-GROUND COMPETITION

Plants use different parts (leaves vs. roots) to compete aboveground (for space and light) and belowground (for nutrients, water, and space) (Casper and Jackson, 1997; Schenk, 2006). The effects of belowground competition are not necessarily additive to those of aboveground competition (Wilson, 1988) but can be opposing and result in complex interactions (Wilson and Tilman, 1995). Roots of different species may interact so that those of one species may increase or decrease the concentration of different resources available to roots of other species (e.g., Schenk, 2006; Berger et al., 2008). It has been argued that, particularly in low productivity environments, belowground competition for nutrients is likely to be more important than aboveground competition for light in promoting the successful establishment and the persistence of invasive species (Dietz and Edwards, 2006). For instance, Kueffer et al. (2007) showed that belowground competition reduced significantly the growth of native juvenile trees in forests dominated by the invasive tree *Cinammomum verum*.

Despite the potentially different effects of above- and belowground competition on the overall outcomes of resource competition between invasive and native species, the majority of studies in plant invasions have focused on observations of patterns in the aboveground vegetation and only few have examined belowground competition between invasive and native species (e.g., Gorchov and Trisel, 2003; Kueffer et al., 2007; Dehlin et al., 2008). How invasive and native plants compete above- and belowground for limiting resources, how they may alter the resources available to neighboring plants, and how they may alter the allocation of available resources to above- vs. belowground structures or to vegetative vs. reproductive structures in neighboring plants, have been seldom explored. More information is also required on how invasive species are associated with soil microbes, including symbiotic and associated N-fixing bacteria and mycorrhizae, in both high- and low-resource ecosystems (Funk, 2013). Mycorrhizae, in fact, may be important mediators of resource competition among plants (e.g., Hetrick et al., 1992; Bazzaz, 1996; Moora and Zobel, 1996), but information on this topic is scarce (Marler et al., 1999).

COMPARING ALIEN DOMINANT AND NATIVE DOMINANT SPECIES

Dominant species, regardless of their native/alien status, are considered to play a major role in regulating plant community dynamics (Grime, 1998; Smith et al., 2004), as they are responsible for most of the biomass in many communities, even where many transient or subordinate species are present (*sensu* Grime, 1998).

Comparisons of the competitive ability of alien vs. native dominants can be useful to assess the role of resource competition in plant invasions, at different stages of the invasion process. Few studies have however addressed this topic, and have done so mainly by comparing biomass as a measure of the competitive ability of a species, although differences in biomass might not be good indicators of a differential competitive ability given that dominant character of the species being compared. Among the studies addressing this question, Bottollier-Curtet et al. (2013) compared five dominant native species and five invasive species that co-occur along the Garonne River, France, and showed that, over a six-month-period, invasive dominants produced larger above- and belowground biomass compared to native dominants in 73% species pairs, suggesting a superior competitive ability of alien dominants over native dominants. Hovick et al. (2011) compared the competitive ability of two co-occurring dominant wetland species, the invasive *Lythrum salicaria* and the native *Typha latifolia*, by examining the colonizing success of seedlings of species other than the two dominants in monocultures of each dominant species. They found that *L. salicaria* reduced the success of colonizing species to a greater degree than *T. latifolia*, although differences in biomass explained little variation in colonizing success, and suggested that *T. latifolia* suppresses colonization via light reduction while *L. salicaria* does so via below-ground competition.

The potential role of dominance by native species in promoting the successful establishment of alien species has been recently emphasized (e.g., Smith et al., 2004; van Riper and Larson, 2009). In an experiment on a native Kansas grassland in which dominance by C_4 grasses was manipulated (reduced by 25 and 50%), Smith et al. (2004) found that invasion by *Melilotus officinalis* was facilitated in plots dominated by dominant natives, due to their capacity to mitigate stressful environmental conditions, while reductions in dominance by C_4 grasses reduced the establishment of the invader. These authors suggested that dominance is a key characteristic determining the establishment of alien species, depending on whether dominant native species exacerbate resource competition or mitigate stressful conditions (Smith et al., 2004). Similar findings for this species were reported by van Riper and Larson (2009), who showed that *M. officinalis* acted as a weak competitor and had no consistent effects on other species in a wheatgrass (*Pascopyrum smithii*) prairie, while, under suboptimal conditions, it acted as a nursing plant, facilitating the growth of other species.

Since dominance represents an important plant community trait (Grime, 2001; Hovick et al., 2011), additional studies are needed to determine whether native dominants may facilitate or prevent plant invasions by alien dominants; whether differences in the competitive ability of alien and native dominants is key to the successful establishment of alien dominants and whether this competitive advantage is likely to be transient or long-lasting.

ACCOUNTING FOR PHYLOGENETIC RELATEDNESS

Phylogenetic relatedness provides a measure of how much evolutionary history two species share and of their ecological similarity (Webb, 2000), with closely related species expected to have traits more similar than phylogenetically distant species, including traits involved in resource competition. Phylogenetic relatedness should thus be accounted for when one wants to capture differences in competitive ability among species, as this alone can explain part of the observed differences. This can be achieved by comparing phylogenetically related species, which allows minimizing trait differences among species associated with their evolutionary history (Powell and Knight, 2009).

Congeneric comparisons between phylogenetically related invasive and co-occurring native species thus represent an effective way of assessing the role of resource competition in the successful establishment of invasive species. Few studies, however, have examined resource competition between invasive and native congeners (Powell and Knight, 2009; Skálová et al., 2013).

Skálová et al. (2013) compared the effects of resource competition in four *Impatiens* species of different origin and invasive potential in central Europe: the native *I. noli-tangere*, and the aliens *I. glandulifera* (highly invasive), *I. parviflora* (less invasive) and *I. capensis* (potentially invasive). They found that *I. glandulifera* was the strongest competitor, followed by *I. parviflora*, particularly under low soil moisture conditions, while *I. capensis* was sometimes limited by alien competitors. These findings seem to indicate that a high competitive ability is important in determining the invasion success of *Impatiens* species and that invading congeners may outcompete the native *I. noli-tangere*. Powell and Knight (2009), in contrast, did not find any evidence for a superior competitive ability of invasive vs. native congeners. They compared the competitive ability of five *Cirsium* species co-occurring in northern California: the invasive *C. vulgare* and four native species, including the endemic *C. fontinale* var. *fontinale*. Contrary to their predictions, *C. fontinale* competed well even under high nutrient conditions and showed no significant reductions in biomass in competition experiments with *C. vulgare*, suggesting that its restriction to low-nutrient serpentine environments is due to factors other than a poor competitive ability in more productive habitats.

Congeneric comparisons between invasive and non-invasive alien congeners, conversely, can be useful to identify those traits that may confer invasive species with a high competitive ability. Several studies have shown that many invasive species possess higher values of competitively-related advantageous traits compared to non-invasive phylogenetically-related species (e.g., McDowell, 2002; Deng et al., 2004), including a higher N allocation to photosynthesis and N-use efficiency (e.g., Feng et al., 2007, 2008, 2009; Feng, 2008), and a higher specific leaf area (e.g., Grotkopp and Rejmánek, 2007; Feng et al., 2008; van Kleunen et al., 2010; Matzek, 2012), larger root biomass, and fast relative growth rate (e.g., Burns, 2004; Grotkopp and Rejmánek, 2007). These traits, however, are not necessarily good predictors of the successful establishment or persistence of an alien species (e.g., Leishman et al., 2010; Meisner et al., 2011), while studies examining resource competition between invasive vs. native congeners, under a range of environmental conditions, could provide important insights into the mechanisms underlying the successful establishment of invasive species.

RESOURCE COMPETITION AND PHENOTYPIC PLASTICITY

Phenotypic plasticity is the ability of a particular genotype to express a range of phenotypes in response to different environmental conditions (Bradshaw, 1965). High phenotypic plasticity in invasive plants has long been regarded as plant feature that may increase the probability of a species to become invasive (Baker, 1965; Pyšek and Richardson, 2006; Richards et al., 2006; Nicotra et al., 2010; Davidson and Nicotra, 2012). Phenotypic plasticity in functional traits may enhance niche breadth (Bradshaw, 1965; Sultan, 2001; Richards et al., 2006), i.e., the niche space or range of conditions required by a species, and may thus play an important role in the successful establishment of alien species in novel environments and its persistence in a community (Palacio-López and Gianoli, 2011).

A large number of studies have examined whether invasive species are more plastic than non-invasive or native species (e.g., Richards et al., 2006; Skálová et al., 2012), even in low resource environments (Funk, 2008), although contrasting results have been reported (Bossdorf et al., 2008; Davidson et al., 2011; Palacio-López and Gianoli, 2011; Matzek, 2012). Greater plasticity could indicate that (1) plasticity plays an important role in determining the successful establishment of alien species; and/or (2) plastic genotypes within species were selected during the invasion process (see Drenovsky et al., 2012 and references therein). In a recent meta-analysis of 75 phylogenetically related invasive/non-invasive species pairs, Davidson et al. (2011) found that invasive species had significantly higher phenotypic plasticity in a wide variety of morphological and physiological traits than non-invasive species, and they were nearly always more plastic in their response to increased nutrient availability than non-invasive species.

As described in Richards et al. (2006), phenotypic plasticity in an invasive species may be adaptive if it enables a genotype to (1) maintain fitness (fitness homeostasis) in unfavorable environments ("jack-of-all-trades" response to decreased resources), (2) increase fitness in favorable environments ("master-of-some" response to increased resources), or (3) both ("jack-and-master" strategy), i.e., a combination of both strategies, which corresponds to the "ideal weed" described by Baker (1965) and could allow a species to maintain high fitness across a broad environmental range (Mozdzer and Megonigal, 2012). The "master-of-some" strategy provides a mechanism by which higher plasticity of invasive species could enable invasive species to outcompete native species, thus facilitating the persistence of alien species in both low- and high-resource environments (Davidson and Nicotra, 2012; Mozdzer and Megonigal, 2012).

Recent findings show that high plasticity is not necessarily correlated to a higher fitness (e.g., Davidson et al., 2011; Matzek, 2012), and our knowledge of the effects of high plasticity as an important species trait in invasion processes is still limited (Hulme, 2008). Matzek (2012) tested the relative contribution of high trait values and high trait plasticity to relative growth rate (a proxy for fitness) for 10 closely related invasive and non-invasive *Pinus* species, and showed that in responding to higher N supply, superior trait values and not trait plasticity provides the better explanation for the performance of invasive species in a changing environment. Davidson et al. (2011) also showed that, despite invasive species having a higher phenotypic plasticity in 75 invasive and non-invasive species pairs, increases in resources did not result in higher fitness in invasive vs. non-invasive species comparisons.

Whether phenotypic plasticity resulting in higher fitness could be adaptive and/or indeed promote the successful establishment, spread, and long-term persistence of alien species has not been clarified (Daehler, 2003; van Kleunen and Fischer, 2005; Peacor et al., 2006; Richards et al., 2006; Davidson et al., 2011; Davidson and Nicotra, 2012; Matzek, 2012) and the effects of high phenotypic plasticity in both invasive and native plants on competitive interactions between invasive and native species requires further investigation.

ROLE OF RAPID EVOLUTION IN RESOURCE COMPETITION

There is evidence that invasive species may show a capacity to undergo rapid evolutionary changes associated with the novel environmental conditions encountered in the communities where they have become invasive (Thompson, 1998; Sakai et al., 2001; Lee, 2002; Stockwell et al., 2003; Bossdorf et al., 2005, but see e.g., Pahl et al., 2013). For some introduced species, adaptations to the novel conditions encountered in the introduced range resulting in an increased competitive ability may substantially alter competitive interactions between alien and native species over time (Bossdorf et al., 2005) and may play an important role in determining the persistence and the impact of an invader on native communities.

The release from natural enemies may alter competitive interactions via the increased reallocation of resources to reproduction and growth that were previously devoted to defense (e.g., Siemann and Rogers, 2001, 2003). This is the basis for the EICA hypothesis (Blossey and Nötzold, 1995; Willis et al., 2000; Vilà et al., 2003; Callaway and Ridenour, 2004), which has been used to explain why many invasive species often occur at greater densities and have a superior competitive ability in their invasive range compared to native species (e.g., Crawley, 1997; Keane and Crawley, 2002; Pergl et al., 2007).

There is little support for the "full" EICA hypothesis being a major factor in the successful establishment of alien plants (Thompson, 2014), with several studies finding no evidence of an increased performance of invasive species released from their specific herbivores, pathogens or parasites (Maron and Vilà, 2001; Thebaud and Simberloff, 2001; Bossdorf et al., 2004, 2005; Maron et al., 2004, 2014; Franks et al., 2008; Ridenour et al., 2008). An additional mechanism potentially leading to an increased competitive ability in invasive species was proposed by Feng et al. (2009), based on observations that the invasive shrub *Ageratina adenophora* had evolved an increased N allocation to photosynthesis (growth) and a reduced allocation to structural defenses (cell walls) in invasive populations compared to native populations. Moreover, if plants in invasive populations had more intra- than interspecific neighbors, they could evolve a reduced competitive ability (Evolutionary Reduced Competitive Ability; ERCA) that would allow the conservation of resources that would be otherwise required to compete against native species (Bossdorf et al., 2004). These resources could then be used for other processes that may lead to successful invasions, such as allelopathy (Prati and Bossdorf, 2004), developing plastic responses, or improving tolerance to herbivory (Bossdorf et al., 2004).

Not only may alien species respond to the novel conditions encountered in the introduced range, but also native species have the potential to adapt to the conditions created by the introduction of invasive species and evolve a capacity to compete with invasive species (Strauss et al., 2006b; Carroll et al., 2007; Mealor and Hild, 2007). Evolutionary changes leading to the genetic adaptation of invasive species and the co-evolution of invasive and native species may strongly affect resource competition between invasive and native species over time.

As changes in competitive ability may be evolutionary or due to phenotypic plasticity, understanding how resource competition between invasive and native species may change over time requires designing experiments that can identify which traits respond evolutionarily and which show a plastic response (and whether these responses will interact).

A TRANSIENT COMPETITIVE ADVANTAGE?

The temporal component of competitive interactions between invasive and native species is an important topic of research in invasion ecology. Increasing evidence shows that competitive advantages of invasive species over natives may be important only in the initial phases of the invasion process (e.g., Milchunas and Lauenroth, 1995; Claassen and Marler, 1998; Corbin and D'Antonio, 2004; Sala et al., 2007; Goldstein and Suding, 2014). Over time, the competitive ability of invasive species could decrease and ultimately result in the displacement of invasive species by natives (see Thompson, 2014 and references therein) through competitive exclusion (Corbin and D'Antonio, 2004; McGlone et al., 2012), although the dynamics of competitive interactions remain unclear.

Evidence for a superior competitive ability of invasive species over natives may thus have been biased by the design and relatively short-term duration of the majority of competition experiments involving native and alien species. Weiner (1993) emphasized the importance of the time scale in the study of competition, pointing out that the outcome of competition between two species may change over time. The major point is that short-term assessments may not give a good representation of the competitive ability of a species over the course of its development (see Milchunas and Lauenroth, 1995; Claassen and Marler, 1998; Corbin and D'Antonio, 2004; Sala et al., 2007; Goldstein and Suding, 2014, see Weiner, 1993; Trinder et al., 2013).

RESOURCE COMPETITION AND INVASIBILITY

RESOURCE AVAILABILITY AND OTHER ABIOTIC CONDITIONS

Resource competition is dependent on the spatial and temporal distribution of resources, and any change in the availability of limiting resources will inevitably alter the competitive balance between invasive and native species (Alpert et al., 2000). It has been shown that a highly heterogeneous distribution of resources may promote high species richness even in strongly competitive communities (e.g., Planty-Tabacchi et al., 1996; Stohlgren et al., 1999), via increasing niche differentiation, i.e., the use of different forms of a resource (Tilman, 2004; see Glossary).

An important question in invasion ecology is whether high-resource environments or environments characterized by low non-resource environmental stresses are more susceptible to invasions by alien plants compared to low-resource environments. Depending on the physiological amplitude of a species, the presence of a major abiotic stress may (1) prevent plant invasions regardless of the competitive abilities of native vs. alien species; (2) may prevent invasions only in combination with competition from native species; or (3) may slow an invasion but not prevent it (Alpert et al., 2000). If native species in a community were competitively superior over alien species, invasions would be prevented or slowed.

Vitousek et al. (1997) suggested that only few ecosystems are unlikely to be invaded by alien species. Despite difficulties in making robust generalizations on the characteristics of invaded communities (Rejmánek et al., 2005), there is evidence that plant communities differ in their degree of invasibility, i.e., their vulnerability to invasions (Lonsdale, 1999; Davis et al., 2000, 2005; see Glossary). High resource environments and/or environments characterized by low abiotic stresses indeed appear to be more invasible than low-resource environments (e.g., Huenneke et al., 1990; Burke and Grime, 1996; Daehler, 2003; Gross et al., 2005;

Stohlgren et al., 2008; Moles et al., 2012; see Funk and Vitousek, 2007; Funk, 2013), although we have already mentioned a potential bias associated with a larger number of studies in high- vs. low-resource environments (e.g., Kueffer et al., 2007; Funk, 2013).

A lower invasibility in low-resource environments is often attributed to the assumption that native species should possess a competitive advantage over invasive species associated with their being adapted to the growth-limiting conditions characterizing such environments, while alien species have not had the opportunity to adapt to the local environmental conditions at the time of introduction ("the paradox of invasion," Alpert et al., 2000; Sax and Brown, 2000; Daehler, 2003). However, jack-of-all-trade alien species can perform as well as natives under a broad range of environmental conditions, thus high resources or low environmental stresses are not good predictors of successful invasions. Many alien species have, in fact, invaded low-resource environments (Funk and Vitousek, 2007; Funk, 2013), including arid and semi-arid grasslands (Fowler, 1986), serpentines (e.g., Huenneke et al., 1990; O'Dell and Claassen, 2006; Vallano et al., 2012), or coastal dunes (e.g., French, 2012; Gioria and Osborne, 2013). Moreover, low nutrient availability may not affect competition (Kolb and Alpert, 2003) and, in one instance, low resources have been found to even promote invasions (Cleverly et al., 1997; see Funk, 2013).

This apparent paradox has been explained with some of the theories described in this paper, including high phenotypic plasticity, a capacity for rapid evolutionary adaptive changes, the release from enemies, high environmental heterogeneity, or a superior competitive ability characterizing invasive species that are native to species-rich regions where resource competition is high (Sax and Brown, 2000). Moreover, in low-resource environments, any temporary increase in available resources can be disproportionally beneficial to invasive plants (e.g., Hobbs and Mooney, 1991; Dukes and Mooney, 1999; Kolb et al., 2002; Thomsen et al., 2006; Abraham et al., 2009), although the temporal dimension of these effects requires additional investigations.

RESOURCE ACQUISITION vs. RESOURCE CONSERVATION TRAITS

A question that has received increasing attention in invasion ecology is whether invasive species possess more resource acquisition or resource conservation traits, in high vs. low resource environments (Crawley et al., 1996; Funk and Vitousek, 2007; Tecco et al., 2010; Funk, 2013). A superior competitive ability of alien species over that of natives is often associated with a high ability to acquire and retain resources (Tecco et al., 2010), although the traits associated with this ability are strongly habitat-dependent (Theoharides and Dukes, 2007). Successful invaders tend to possess traits associated with rapid resource acquisition and growth, including nutrient-rich leaves, with a high specific leaf area, and a short lifespan, in high resource environments (Burns, 2004, 2006; Blumenthal, 2005; Leishman and Thomson, 2005; Rejmánek et al., 2005; Grotkopp and Rejmánek, 2007; Leishman et al., 2007; Feng, 2008; Feng et al., 2008; van Kleunen et al., 2010; Matzek, 2012), while in low-resource environments, invasive species vary significantly in their strategies to cope with low resource availability, possessing either traits indicative of resource conservation or resource acquisition strategies (Funk, 2013). In particular, invasive species appear to use nutrients more efficiently than co-occurring native species in low-nutrient soils, while in light-limited systems, invasive and native species are similar in their water use efficiency (Funk, 2013).

The life form of the invaders may affect the results of studies addressing this research question. In an investigation comparing functional traits in native and alien species of central-western Argentina, across contrasting ecosystem types and management regimes, Tecco et al. (2010) showed that woody alien species possessed significantly more acquisitive sets of attributes than native species, while they did not detect any significant difference in trait syndrome (acquisitive vs. conservative) between herbaceous alien and native species.

It is worth noting that acquisitive vs. conservative strategies or syndromes in low-resource ecosystems are not necessarily incompatible, and that enhanced resource acquisition and the sparing use of those resources in biomass production could arguably go hand in hand, and differences with natives could be dependent upon the "opportunistic" response with a higher capacity of invasive species to exploit pulses or enhanced resource levels being important (see also Grime and Hunt, 1975 on variation in relative growth rate). Future research should examine the extent to which differences in functional strategies (acquisitive vs. conservative) in invasive and native species may help predict the outcomes of competitive interactions between invasive and native species.

DISENTANGLING RESOURCE COMPETITION FROM OTHER MECHANISMS

COMPETITION, RECRUITMENT LIMITATION

Recruitment limitation in both alien and native species may affect the outcomes of resource competition and resource competition dynamics (e.g., Hamilton et al., 1999; French et al., 2011; Gioria et al., 2012). In his neutral theory of ecological equivalence of species in a community, Hubbell (2001, 2006) proposed that recruitment limitation can delay competitive exclusion associated with asymmetric competition. In a theoretical study on plant competition for space, Hurtt and Pacala (1995) showed that competitively inferior species can coexist with dominant, competitively superior species, when the dominant species is recruitment limited. Thus, in the absence of niche differentiation (see Glossary), the outcomes of competition should mainly depend on differences in the competitive ability of alien vs. native species, with competitively superior species ultimately excluding competitively inferior species. Recruitment limitation in invasive species that are better competitors than natives should thus delay competitive exclusion of natives, while recruitment limitation in native species should exacerbate the effects of competition with competitively superior invasive species. This is consistent with the results of experimental studies showing that recruitment limitation in native species exacerbated the competitive effects of invasive species (Tilman, 1997; MacDougall, 2004), while Seabloom et al. (2003) showed that dominance patterns by alien annuals were likely caused by recruitment limitation of native perennial species rather than by a superior competitive ability of alien species in a California grassland community.

The importance of recruitment limitation relative to that of resource competition in determining the successful establishment of alien species and in the persistence of invasive species in a community deserves further investigations, as recruitment limitation in native species may increase the invasibility of native communities regardless of the competitive ability of the introduced alien species (Hamilton et al., 1999; Turnbull et al., 2000; French et al., 2011; Gioria et al., 2012).

NICHE vs. FITNESS DIFFERENCES

Niche differences reflect differences in resource use or response deriving from long-term competitive interactions among the species present in a community (Bazzaz, 1996; see Glossary). In contrast, fitness differences reflect differences in competitive ability (e.g., Tilman, 1988), in fecundity, or in the susceptibility to predators and pathogens (see MacDougall et al., 2009), and can be estimated by differences in growth rate (Adler et al., 2007). Niche and fitness differences have opposite effects on the outcomes of competition (Chesson, 2000) and may strongly affect the importance of resource competition in the successful establishment, spread, and persistence of alien species, although their importance relative to that of resource competition requires further investigations.

MacDougall et al. (2009) proposed an interesting framework to unify previous theories on coexistence between alien and native species along a fitness and niche differences axis. Niche differences between alien and native species may facilitate the establishment of alien species (MacDougall et al., 2009), by allowing an alien species to avoid resource competition, and may favor coexistence (Adler et al., 2007). In contrast, in the absence of niche differences, fitness differences will lead to the competitive exclusion of species with a comparatively low average fitness. This framework also encompasses the Empty Niche Hypothesis (e.g., Stachowicz and Tilman, 2005), which postulates that the presence of empty niches, i.e., niches not occupied by any native species, may promote invasions by niche-differentiated alien species due to the incomplete use of resources by native species. Thus, even if an alien species was a poor competitor, it could establish and, ultimately, become invasive in the presence of empty niches.

INDIRECT COMPETITION

Besides competition for resources, other types of interactions (e.g., indirect competition and allelopathy) may affect the establishment, spread, and persistence of alien species in invaded communities. Indirect competition includes competition for shared pollinators and apparent competition (see White et al., 2006). Competition for shared pollinators often results in a reduced visitation of pollinators to native species associated with the presence of an alien species (e.g., Brown et al., 2002; Moragues and Traveset, 2005; Munoz and Cavieres, 2008; Kandori et al., 2009; Morales and Traveset, 2009; Palladini and Maron, 2013) and may be exacerbated by the dominance of invasive species in a community (Bjerknes et al., 2007; Morales and Traveset, 2009). Despite an increasing interest in this type of competition, studies assessing the importance of resource competition relative to that of competition for shared pollinators are scarce. Among these studies, Palladini and Maron (2013) showed that, although the invasive perennial forb *Euphorbia esula* reduced substantially pollination visitation to native annual *Clarkia pulchella*, native plants were not pollen-limited, suggesting that resource competition was more important than apparent competition in conferring *E. esula* a competitive advantage over *C. pulchella*.

Apparent competition between plants occurs when one species alters the abundance or the distribution of consumers and thus the consumption of other species (Holt, 1977; Holt and Kotler, 1987; Connell, 1990). More specifically, apparent competition may occur (1) when a species provides a consumer with a non-food resource, e.g., shelter, allowing the consumer population to increase and spread, with subsequent negative effects on the native species, or (2) when both plant species provide a food-resource to a food-limited consumer (e.g., Sessions and Kelly, 2002; Orrock et al., 2008; Dangremond et al., 2010; Recart et al., 2013).

Relatively few studies have examined the role of apparent competition in promoting plant invasions, although the interest in this type of competition has increased. There is evidence that apparent competition between alien and native species have significant negative consequences for native species (Sessions and Kelly, 2002; Orrock et al., 2008; Dangremond et al., 2010; see White et al., 2006 for a review), although its effects appear to be strongly context-dependent (e.g., Orrock and Witter, 2010; Mattos et al., 2013; Recart et al., 2013). Such negative effects of apparent competition could contribute to reduce the competitive ability of native species over that of invasive species. The presence of alien species may, however, have positive effects on the competitive ability of native species by reducing the pressure of generalist herbivores on native species. Recent studies (Jacquemart et al., 2013) and meta-analyses (Parker and Hay, 2005; Parker et al., 2006) indicate that some generalists have a preference for alien plant hosts over native plants, while some alien plants may negatively impact on the survival of generalist herbivores (Tallamy et al., 2010), and may thus benefit native species, altering the competitive balance between alien and native species. How apparent competition may affect or interact with resource competition in determining the establishment of alien species remains unclear and requires additional investigations.

ALLELOPATHY

Allelopathy can be defined as the effect of one individual on its neighbors associated with the release of chemical compounds from roots, shoots, leaves, or flowers (Rice, 1984, see Glossary). The Novel Weapon Hypothesis postulates that the invasiveness of certain alien species could depend on their ability to produce secondary metabolites that are evolutionarily novel in their introduced range and that interfere with native plants, microbes, pathogens, or generalist herbivores and reduce the growth of native plants (e.g., Callaway and Aschehoug, 2000; Bais et al., 2003; Hierro and Callaway, 2003; Callaway and Ridenour, 2004; Callaway et al., 2004; Pisula and Meiners, 2010; Uddin et al., 2014). The production of allelochemicals generally has effects that are greater in a species' introduced range than in its native range (e.g., Callaway and Aschehoug, 2000; Bais et al., 2003; Hierro and Callaway, 2003; Callaway and Ridenour, 2004; Callaway et al., 2004, 2008; Prati and Bossdorf, 2004; see Inderjit et al., 2011 and references therein for a discussion on evolutionary changes in allelochemical effects).

While a capacity to produce chemical defenses against competitors has been viewed as a factor potentially conferring a species with a competitive advantage over neighboring species, recent evidence shows that increases in the production of allelochemicals in response to intense resource competition may substantially reduce the growth of the same species and increase their palatability to herbivores, with potential negative effects on their ability to compete for resources (Rasher and Hay, 2014). The full ecological implications of allelopathy on resource competition between alien and native species remain unclear. Future research on this topic must, however, be reconciled with the well-known difficulties associated with separating the effects of resource competition from those of allelopathy in natural systems (see Inderjit and del Moral, 1997).

FUTURE RESEARCH DIRECTIONS

GLOBAL CHANGE AND RESOURCE AVAILABILITY

Global environmental changes, such as climate change, increasing atmospheric CO_2, and atmospheric N deposition, will affect the spatio-temporal distribution and dynamics of the resources available to plants (Wedin and Tilman, 1996; Dukes, 2000, 2002b; Smith et al., 2000). Such changes will inevitably affect resource competition between alien and native species (e.g., Vitousek, 1994; Vitousek et al., 1996; Dukes and Mooney, 1999; Dukes, 2000, 2002b; Novoplansky and Goldberg, 2001; James et al., 2006; Bradley et al., 2009; Firn et al., 2010).

Competition experiments can provide important insights into the effects of global environmental changes on resource competition between alien and native species, although, to date, expected changes in competitive interactions among species have been mainly based on individual species responses (see Goldstein and Suding, 2014 and references therein). An interesting study on the effects of climate change on competitive interactions between alien and native species is that of Goldstein and Suding (2014), who examined changes in resource competition between alien grasses and California coastal sage scrub species associated with projected changes in rainfall patterns in additive competition experiments, under three rainfall treatments: (1) frequent small events, (2) infrequent large events, and (3) infrequent small events. Rainfall amount and frequency altered competitive interactions between California coastal sage scrub and grasses. In the first year, the competitive effect of annual grasses on shrub seedlings was strongest under treatment (1), while in the second year, the established shrubs started exerting strong competitive effects on grasses, particularly under treatment (3) with a low total rainfall. These findings suggest that reductions in both rainfall frequency and total rainfall may alter plant community composition and invasion dynamics via alterations in competitive interactions between alien grasses and native species.

Increasing levels of N deposition associated with anthropogenic activities are expected to favor the establishment and long-term persistence of invasive species (e.g., Dukes and Mooney, 1999; Bobbink et al., 2010; Vallano et al., 2012). The strong positive growth and competitive response of many invaders to N addition (e.g., Huenneke et al., 1990; Witkowski, 1991; Milchunas and Lauenroth, 1995; Burke and Grime, 1996; Daehler, 2003; Lowe et al., 2003; Leishman and Thomson, 2005) suggest that N deposition may increase the competitive ability of invasive species vs. that of natives, particularly in low-nutrient environments, where native species are adapted to nutrient-deficient soils (e.g., Huenneke et al., 1990; Burke and Grime, 1996; Kolb et al., 2002; Lowe et al., 2003; Thomsen et al., 2006; Vallano et al., 2012).

Increasing concentrations of atmospheric CO_2 are also expected to impact on resource competition between alien and native species. Evidence shows that elevated CO_2 stimulates photosynthetic carbon gain and net primary production, improves nitrogen use efficiency, and decreases water use, thus removing some moisture constraints (e.g., Ainsworth and Rogers, 2007; Leakey et al., 2009). In competition-free systems (experiments conducted using monocultures), invasive plants seem to respond strongly to increases in CO_2, in the short-term, but their response in competitive systems could be reduced (Bazzaz and McConnaughay, 1992; Dukes, 2000). How projected global environmental changes may affect resource competition between alien and native species in competitive systems still remains unclear and deserves further investigation (Dukes, 2002a; Vallano et al., 2012; Goldstein and Suding, 2014).

ALTERATIONS IN RESOURCE AVAILABILITY BY ESTABLISHED ALIEN SPECIES AND SECONDARY INVASIONS

There is strong evidence that many invasive species are capable of altering the levels of available resources in invaded ecosystem (e.g., Vitousek et al., 1996; Lindsay and French, 2004, 2005; Ehrenfeld, 2010; Vilà et al., 2011; Pyšek et al., 2012). Plant invasions may do so by altering the composition of native communities and patterns of dominance among plant functional types, including herbaceous vs. woody plants, C_3 vs. C_4 species, or nitrogen-fixing vs. non nitrogen-fixing species. These changes can strongly affect the distribution and dynamics of soil nutrients and other resources (e.g., Vitousek et al., 1987; Fogarty and Facelli, 1999; Gill and Burke, 1999; Ehrenfeld, 2010), by alterations in nutrient availability associated with the introduction of nitrogen-fixing invaders (Vitousek and Walker, 1989; Gioria et al., 2011), increased light availability via a reduction in the biomass of resident species (Flory and Bauer, 2014), or reductions in the amount of available water by deeply-rooted invaders, such as salt cedar *Tamarix* (Vitousek and Walker, 1989).

Invasive species may also alter the soil biota through plant-soil feedbacks (e.g., Kulmatiski et al., 2008; Suding et al., 2013), with potential negative effects on native species, such as those caused by the introduction of soil-borne pathogens, herbivores, or parasites, or positive effects, such as those associated with increases in mycorrhizal fungi or nitrogen fixing bacteria (Klironomos, 2002; Callaway et al., 2004, 2008; Ehrenfeld, 2010; Gioria and Osborne, 2013). In contrast, plant-soil-feedbacks may be beneficial to the invader itself (e.g., Reinhart and Callaway, 2006; Bever et al., 2010; Smith and Reynolds, 2012; but see Levine et al., 2006; Shannon et al., 2012; Suding et al., 2013). Shannon et al. (2012) showed that plant-soil feedbacks may change with modifications in competitive interactions between invasive and native species, as an invasion process progresses.

Changes in the availability of resources associated with plant invasions may thus create conditions that may either increase or decrease the competitive ability of invasive species vs. that of native or other alien species. How these changes will affect resource competition between alien and native species is a key to improving our understanding of the long-term implications of plant invasions on native communities. Moreover, such changes could create conditions that facilitate secondary invasions, i.e., the establishment of other alien species in a community (e.g., Gioria et al., 2011). The study of secondary invasions could provide important insights into competitive interactions among alien dominant species and resource competition dynamics, and represents an important topic that has so far received little attention.

ISSUES AND GUIDELINES FOR FUTURE STUDIES

Future research studies must address a number of deficiencies that have characterized many competition experiments in plant invasion ecology and that might have hindered our capacity to predict the role of resource competition in plant invasions. Throughout this review, we have pointed out a number of research needs on the role of resource competition in plant invasions, including that for more direct measurements of the competitive ability of invasive and native species (see also Trinder et al., 2013), under a broad set of environmental conditions representative of those that may be encountered in the field, as well as testing the interactive effects of multiple abiotic conditions (see Nernberg and Dale, 1997; Sammul et al., 2000), given that plants are typically subjected to more than one environmental source of stress and that the response to multiple stress factors may not be predictable on the basis of each applied individually (Mittler, 2006).

Another issue characterizing competition studies on plant invasions is that many pair-wise competition experiments have compared the competitive ability of alien and native species possessing contrasting life forms, such as annuals vs. perennials (Claassen and Marler, 1998; Groves et al., 2003; Abraham et al., 2009; Mangla et al., 2011a), forbs vs. grasses (Callaway and Aschehoug, 2000; Sharma et al., 2010), or herbaceous vs. woody species (Eliason and Allen, 1997; Bottollier-Curtet et al., 2013), with potential significant effects on the outcomes of competitive interactions. Clearly, different life forms may be associated with variations in the timing and magnitude of resource use, regardless of their native/alien status. For instance, the dominance and suppression of winter and summer annuals does not depend on differences in the competitive abilities among these life forms, but mainly on differences in the timing of soil disturbance (Bazzaz, 1996). Similar considerations pertain to comparisons of the competitive ability of annual vs. perennial species or that of herbaceous vs. woody species. Future experiments should thus account for differences in the type and timing of resources required by different life forms for both alien and native species, since competition experiments where different life forms are sown synchronously might fail to represent realistic competition dynamics in the field and possibly overestimate the effects of resource competition and the competitive ability of invasive species.

Resource competition is a dynamic process (Trinder et al., 2013) and is strongly linked to resource availability dynamics. Competitive interactions between invasive and native species should thus be examined over time and should be carried out at different stages of development, for both invasive and native species (e.g., Weiner, 1993; Mangla et al., 2011b; Trinder et al., 2013). It has been suggested that, after an initial phase where pre-adapted species become dominant, during a second phase, they can then spread into low resource environments due to shifts in life history traits either via plastic responses or adaptive evolution or both (Dietz and Edwards, 2006). Future research in plant invasions should thus examine how resource competition may change over time due to phenotypic plasticity and/or the ability of alien species to evolve and adapt to the new conditions experienced in their introduced range, during each phase of the invasion process, accounting for the temporal scale of these processes.

Interpreting the outcomes of resource competition strongly depends on the way competition is measured (Goldberg et al., 1999; Weigelt and Jolliffe, 2003; Freckleton et al., 2009). While competition intensity, i.e., the absolute magnitude of competitive effects (see Glossary; Grace, 1991, 1995) has been examined extensively, the importance of competition, i.e., the effects of resource competition on community composition or community dynamics relative to those of other types of interaction (see Glossary; e.g., Goldberg, 1994), has been assessed less frequently. While intensity refers mainly to the present process of competition, its importance also reflects the results of past competition (Welden and Slauson, 1986). Assessing the role of resource competition in plant invasions requires information on both its intensity and its importance relative to that of other mechanisms or processes.

Phylogenetic and niche differences between alien and native species may confound the effects of resource competition and should be accounted for when predicting the outcomes of resource competition among species. Phenological differences between alien and native species should also be accounted for when examining the role of resource competition in plant invasions, as they may strongly affect competitive interactions, particularly at the early stages of plant development, and could explain why some invasive species may not be strong competitors if they can take advantage of temporal windows of opportunity when competitive interactions are weak or non-existent (**Figure 1**).

Coevolutionary responses among competing plants have been generally neglected (Leger and Espeland, 2010; Lankau, 2012). However, assessments of the reciprocal evolutionary responses of invasive and alien species after the introduction of an invasive species could improve substantially our capacity to predict resource competition dynamics (Strauss et al., 2006a; Mealor and Hild, 2007) and could provide some insights into why some initially successfully invaded locations are subsequently replaced by native or other alien species, as has been observed in some instances (Gioria et al., 2011; Thompson, 2014).

Future studies should also examine more extensively how clonal integration, i.e., resource sharing among interconnected ramets, or plant parts (see Glossary; e.g., Alpert and Mooney, 1986; Alpert, 1996; Liu et al., 2006; Xu et al., 2012), could affect the competitive ability of clonal invaders. Clonal integration may buffer the effects of the heterogeneous distribution of soil resources (Hutchings and Wijesinghe, 1997) and has been shown to affect the response of invasive clonal species to variations in light (Xu et al., 2012), water (You et al., 2013), and

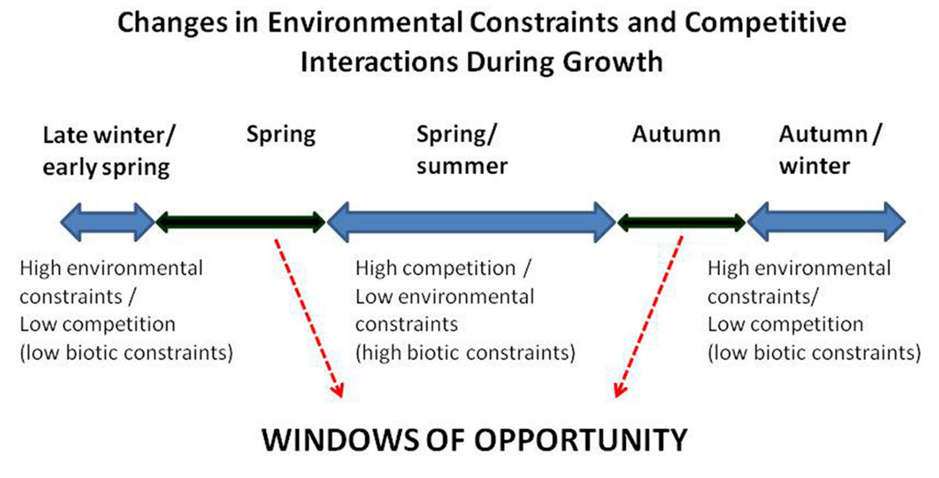

FIGURE 1 | Diagrammatic representation of changes in environmental constraints and competitive interactions during vegetative growth and development over the course of a year. Note that in spring and, to some extent, in autumn, competition will be low, although there could be high to moderate environmental constraints to growth and development that could impact on any introduced alien plant species. At other times, competitive interactions will be high, with the possibility of biotic constraints associated with near optimal environmental conditions during the main growth period. The "windows of opportunity", designated by black arrows, represents periods of reduced competition, with the spring "window" likely to be greater than the autumn "window", particularly in cases with low vegetation cover or where the onset of growth-limiting environmental constraints occur rapidly. Establishment in the autumn "window" will be severely constrained by gradually decreasing temperatures and day length. The maintenance of a long-lived canopy well into the autumn "window", a feature of many invasive plant species, will also reduce recruitment or end-of-season growth and seed germination of native species. Even if some growth or seed germination does occur, these individuals are unlikely to overwinter.

nutrient availability (You et al., 2014). Despite several invasive species being clonal, the role of clonal integration on competitive interactions for resources has been examined only recently (Wang et al., 2008; You et al., 2013), with studies showing that it can promote invasiveness under heterogeneous conditions (You et al., 2013, 2014) via its effects on growth, biomass allocation, and photosynthetic efficiency (Wang et al., 2008; Xu et al., 2012; You et al., 2013) and facilitate colonization under competitive situations (Xiao et al., 2011).

It is beyond the scope of our review to propose a detailed sampling framework or to describe in detail the drawbacks and advantages of experimental designs for assessing the importance and intensity of resource competition between alien and native species (see Trinder et al., 2013 for a discussion of the issues characterizing assessments of resource competition among plants). More complex experiments, over temporal scales that allow assessing competition dynamics, are needed to improve our capacity to characterize the role of resource competition in plant invasions, as well as to predict the long-term implications of the introduction of alien species. A common sampling protocol to assess competition dynamics and compare the competitive ability of alien vs. native species, based on standardized measures of competition importance, competition intensity, competitive ability, or competition effects (e.g., Grace, 1995; Goldberg et al., 1999; Brooker and Kikividze, 2008; Freckleton et al., 2009; Damgaard and Fayolle, 2010), would allow comparisons the results of multiple studies in multiple regions and ecosystem types, including comparisons of the competitive ability of selected invasive species, across ecosystem types and geographical regions, thus providing insights into the effects of phenotypic plasticity and evolutionary changes on the importance of resource competition in plant invasions.

CONCLUSIONS

Resource competition has long been regarded as a major determinant of the successful establishment and spread of alien species and their long-term persistence in invaded communities, although its importance relative to that of other mechanisms remains unclear. As resource competition is a dynamics process, its role in plant invasions will inevitably change over time, not only due to changes in available resources associated with disturbances, global environmental changes, or changes promoted by the invaders themselves, but also due to plastic responses and evolutionary changes that may occur during invasion processes in both invasive and native species. In this review, we highlighted the most pressing research needs in this field and described a range of factors that may confound our capacity to determine the importance of resource competition in plant invasions, including phenological differences resulting in competition avoidance, niche and fitness differences, phylogenetic relatedness, recruitment limitation, indirect competition, or allelopathy. Improving our understanding of the role of resource competition in plant invasions and its dynamics does not only represent a key ecological question but is essential to predicting the long-term impacts of plant invasions and of how they may interact with other global environmental changes.

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GLOSSARY

Allelopathy: the negative effects of one individual on its neighbors associated with the release of chemical compounds from roots, shoots, leaves, or flowers (Rice, 1984).

Apparent competition: an indirect type of competitive interaction that occurs when one species alters the abundance or the distribution of consumers and thus the consumption of other species (Holt, 1977). Apparent competition may occur when a species provides a consumer with a non-food resource, e.g., shelter, allowing the consumer population to increase and spread, with subsequent negative effects on the native species, or when both plant species provide a food-resource to a food-limited consumer (Dangremond et al., 2010).

Asymmetric competition: an unequal division of resources among competing plants (Freckleton and Watkinson, 2001). It occurs where some individuals or some species remove a disproportionately large amount of resources (Freckleton and Watkinson, 2001). Where asymmetric competition occurs due to differences in size that confer an initial size advantage, the competitive effect is larger than the difference in size, meaning that if an individual is twice the size of another individual, the competitive effect must be more than twice or the larger individual take up more than twice the resources available (Weiner, 1993).

Clonal integration: resource sharing among interconnected ramets (Alpert and Mooney, 1986).

Competition importance: the relative impact of resource competition, among other processes, on plant fitness, community composition or population dynamics (Welden and Slauson, 1986). Competition importance is an ecological concept.

Competition intensity: the degree to which resource competition by neighboring individuals reduces the performance of an individual (or species) below a value when no neighbors are present (Welden and Slauson, 1986). Competition intensity is a physiological concept.

Competitive ability: the ability of a species to acquire limiting resources and/or a capacity to cope with low resource levels or to reduce the availability of resources to its neighbors. The competitive ability of a species has two components: (1) **competitive effect**: the ability to take up resources and thereby reduce the amounts available for other plants (Goldberg, 1990); (2) **competitive response**: the ability to perform well even though resource levels are reduced by the competitors (Goldberg, 1990).

Disturbance: the partial or total destruction of the plant biomass that can arise from the activities of herbivores, pathogens and humans (trampling, mowing, and plowing), and from phenomena such as wind damage, frosts, droughts, soil erosion, and fire (Grime, 2001).

Indirect competition: complex competitive interactions involving more than two species, resulting from the effects of one species on a third species via effects on a second species (e.g., White et al., 2006).

Invasive alien species: a subset of **alien** species, i.e., species that have been introduced either intentionally or unintentionally outside their native geographical range, which have become **naturalized** plants that produce reproductive offspring, i.e., have formed self-sustaining populations without direct human intervention, and have become invasive, i.e., are found often in very large numbers, at considerable distance from the parent plants, thus having the potential to spread over a large area (Richardson et al., 2000). Approximate scales: >100 m in <50 years for species spreading by seeds and other propagules (for dioecious taxa that rely exclusively on seeds for reproduction, this applies only after the introduction of both sexes); >6 m in 3 years for species spreading by roots, rhizomes, stolons, or creeping stems (Richardson et al., 2000).

Invasibility: the susceptibility of a community to the colonization and establishment of introduced alien species (Lonsdale, 1999). Invasibility can be quantified as the probability of successful establishment per arriving propagule (Davis et al., 2005). Invasibility describes a community's potential to be colonized, while the realization of that potential is dependent on the presence and abundance of propagules (Davis et al., 2005).

Niche differentiation: differential resource use or response resulting from long-term competitive interactions between species in a community (Bazzaz, 1996).

Resource competition: a negative interaction between individuals or species associated with a requirement for shared limiting resources resulting in a reduction in one or more fitness components at the individual level or at the population level (Goldberg et al., 1999). From a functional point of view, competition can be regarded as an alteration of the processes of (1) "acquisition" of resources, (2) their "allocation" to different parts, and (3) the "deployment" of these parts in space (Bazzaz, 1996), by neighboring individuals.

Resource: consumable or depletable "supply factors" that are required by plants for maintenance, growth, and reproduction (e.g., Harper, 1977), including light, water, nutrients, oxygen, and CO_2.

Non-resource condition: include "non-consumable" factors, such as temperature. Some factors, such as light, can be both resource and non-resource conditions (Bazzaz, 1996).

Stress: physical, chemical, and biological constraints that restrict photosynthetic production. These include shortage of light, water and mineral nutrients, or suboptimal temperatures (Grime, 2001).

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Overcoming barriers to seedling regeneration during forest restoration on tropical pasture land and the potential value of woody weeds

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Combating the legacy of deforestation on tropical biodiversity requires the conversion to forest of large areas of established pasture, where barriers to native plant regeneration include competition with pasture grasses and poor propagule supply (seed availability). In addition, initial woody plants that colonise pasture are often invasive, non-native species whose ecological roles and management in the context of forest regeneration are contested. In a restoration experiment at two 0.64 ha sites we quantified the response of native woody vegetation recruitment to (1) release from competition with introduced pasture grasses, and (2) local facilitation of frugivore-assisted seed dispersal provided by scattered woody plants and artificial bird perches. Herbicide pasture grass suppression during 20 months caused a significant but modest increase in density of native woody seedlings, together with abundant co-recruitment of the prominent non-native pioneer wild tobacco (*Solanum mauritianum*). Recruitment of native species was further enhanced by local structure in herbicide-treated areas, being consistently greater under live trees and dead non-native shrubs (herbicide-treated) than in open areas, and intermediate under bird perches. Native seedling recruitment comprised 28 species across 0.25 ha sampled but was dominated by two rainforest pioneers (*Homalanthus novoguineensis*, *Polyscias murrayi*). These early results are consistent with the expected increase in woody vegetation recruitment in response to release from competitive and dispersive barriers to rainforest regeneration. The findings highlight the need for a pragmatic consideration of the ecological roles of woody weeds and the potential roles of "new forests" more broadly in accelerating succession of humid tropical forest across large areas of retired agricultural land.

Keywords: rainforest, regrowth, seed dispersal, novel ecosystem, old field, plant invasion

INTRODUCTION

Approximately half of the world's tropical biomes have been subjected to some form of clearing (Asner et al., 2009). One of the major drivers behind deforestation of tropical forests is clearing for agricultural practices (Achard et al., 2002). Consequently, large tracts of continuous rainforest have been converted to fragmented patches of remnant forest and secondary regrowth, situated amongst mosaics of agricultural land and cattle pastures (Turner and Corlett, 1996). This process threatens global biodiversity, and causes increased global carbon emissions and changes in ecosystem functioning (Bradshaw et al., 2008). In some tropical forest landscapes, areas that were initially cleared for pasture and cattle grazing are eventually abandoned, due to declining productivity of pasture grasses, ongoing soil degradation, invasion of unpalatable grasses and changing socio-economic incentives (Hobbs and Cramer, 2007; Grau and Aide, 2008).

Forest recovery may subsequently occur in these retired tropical pastures, but several ecological factors act to reduce colonization by rainforest plants, potentially leaving the landscape in a state of arrested succession (Holl et al., 2000; Kanowski et al., 2009). Competition plays an important role in these dynamics, because a persistent cover of pasture grasses and herbs can limit forest regeneration following the removal of grazing livestock, by restricting micro-climatic conditions required for seed germination and the access of newly recruited woody seedlings to light, soil moisture or nutrients (Holl, 2002). Additionally, recovery of tropical forests is also often limited by a lack of propagule (seed) supply, because the seeds of many rainforest tree and shrub species have short-duration viability and are quickly exhausted from the soil seed bank during prolonged land use (Uhl, 1987; Holl et al., 2000). Furthermore, they are typically produced within fleshy fruits, so that their seed dispersal is mediated by frugivorous vertebrates that do not frequently visit open pasture (Da Silva et al., 1996; Wunderle, 1997).

Rapid reforestation over areas that are sufficiently large to be ecologically useful requires management interventions to reduce these barriers to regeneration. A variety of such interventions have been explored in recent years by an emerging cohort of restoration practitioners (Shoo and Catterall, 2013). For example, suppression of pasture grasses through various methods can in some circumstances promote reestablishment of woody vegetation (Shoo and Catterall, 2013). Additionally, the presence of isolated paddock trees potentially attracts seed dispersing animals which may move from forest to pasture and thereby facilitate seedling establishment (Guevara et al., 2004; Manning et al., 2006). Installation of artificial perches may similarly encourage seed rain, although subsequent seedling recruitment appears limited when pasture grasses are present (Holl, 1998; Shiels and Walker, 2003; Graham and Page, 2012). By contrast, canopy shade provided by live established paddock trees could competitively limit the growth of pasture grasses and herbs as well as potentially improving micro-climatic and soil conditions toward a more favorable environment for rainforest seedling recruitment (Rhoades et al., 1998; Manning et al., 2006). However, for these and other potential management interventions, there has been only limited systematic assessment of their effectiveness (Shoo and Catterall, 2013). A further dimension of management complexity occurs because non-native invasive plants are often the first and most abundant woody species recruited into retired pasture; these could act as recruitment facilitators for native forest seedlings by both attracting frugivores and shading the ground, but their treatment in restoration is controversial because of the possibility that they may also have negative effects on forest recruitment (D'Antonio and Meyerson, 2002; Kanowski et al., 2008; Davis et al., 2011).

Here we investigate methods for encouraging recruitment of rainforest seedlings in retired tropical pasture by removing or manipulating certain ecological barriers. We use a realistically scaled management experiment established in the Wet Tropics uplands of north eastern Australia to quantify the short term response of native woody seedling recruitment to: (1) herbicide-induced release from competition with introduced pasture grasses; and (2) local facilitation provided by elements of habitat structure, specifically scattered trees, shrubs, and artificial bird perches. We test whether rainforest seedling recruitment increases when competitive and dispersive barriers to regeneration are reduced. We also describe the extent and pattern of co-recruitment by non-native invasive woody plants, and discuss the findings in the context of current understanding of old field restoration in the tropics, with a particular emphasis on the contentious role of woody weeds in efforts to reinstate forest over large areas.

METHODS

STUDY AREA

An experimental restoration project ("Kickstart Pasture Conversion Trials") was established in November 2011 in areas adjoining the Mt Hypipamee/Upper Barron section of the Wet Tropics World Heritage Area, on the Atherton Tableland, north eastern Australia. The native vegetation is complex notophyll to mesophyll vine forest (Tracey, 1982). Landscape vegetation cover at the time of this study was a mosaic of remnant forest, substantial areas of livestock pasture (from which forest was cleared mainly in the first 5–6 decades of the twentieth century), and regrowth forest of varying ages. Pasture areas were mainly used for cattle grazing, and by the end of the century were dominated by non-native tropical grasses, such as signal grass (*Urochloa decumbens*) and pasture legumes, with guinea grass (*Megathyrsus maximus*) and setaria (*Setaria sphacelata* var. *anceps*) also widely established, together with a wide variety of other planted and invasive species.

So far, three kickstart pasture conversion sites have been established, each on red basaltic soil in an area of retired pasture which slopes steeply northwards to an east-flowing gully bordering a large area of conserved rainforest. In this paper we restrict most analyses to two sites from which the longest data time series is available (CloudlandE and CloudlandW; **Figure S1**), located on a single property (Cloudland: 17° 27′ 59″ S, 145° 32′ 28″ E, 875 m elevation), and separated by approximately 400 m which includes a strip (150 m wide) of restored rainforest that was planted in 2007. Limited shorter-term data are also presented from the third, later-established, site (Ringtail: 17° 27′ 59″ S, 145° 32′ 28″ E, 821 m elevation, some 3 km from Cloudland). The Cloudland property experienced cycles of partial clearing and regrowth since before the 1940s, and was partly cleared and grazed in the 1980s and 1990s, until 2005 when the entire property was destocked followed by ongoing exclusion of all grazing livestock. At the commencement of the study, pasture grass cover remained dense and was taller (often 0.5–1.0 m) than when grazed, and was variably intermixed with scattered colonising woody plants 2–8 m tall, comprising either native rainforest or non-native invasive species, together with occasional other small patches of low (<1.5 m tall) shrub or vine growth. Nearby weather stations indicate an average annual rainfall of 1467 mm, with 75% of the total falling between December and April (2001–2013, 2.6 km away at 031184 McKell Road Alert) and average monthly minimum and maximum temperatures of 14.7 and 25.4°C respectively (1961–1990, 16.5 km away at 031029 Herberton Mowbray Rd).

DESIGN OF EXPERIMENTAL RESTORATION TREATMENTS

Each site contained two types of plot 8–200 m apart within the retired pasture, each plot being a square 80 m by 80 m (0.64 ha) with one side abutting the rainforest edge: (1) the "Works plot" within which all experimental management works took place; and (2) the "Control plot," a similar delineated area of retired pasture which remained untreated (**Figures S1**, **S2**). Management interventions in each Works plot were of two types: (1) grass and herb suppression; and (2) installation of bird-attracting structures. In addition, we also investigated (3) the influence of pre-existing trees and shrubs.

Grasses, herbs and other low-growing pasture-associated plants were suppressed with repeated herbicide applications, at a frequency which depended on weather conditions and observed grass or herb regrowth, as judged during regular site inspections (in general, grass and herb growth was slower during the cooler winter months). To reduce external influences, a 5.0 m wide buffer around the perimeter of the Works plot was also treated with herbicide. Herbicides were either glyphosate (which has a broad spectrum action on all types of plant) or the grass-selective Fusilade (fluazifop-p butyl) and Verdict (haloxyfop-R-methyl).The initial herbicide treatment in each Works plot aimed to achieve comprehensive glyphosate coverage of all ground-level plants, with no attempts to locate or protect small native forest seedlings, very few of which were apparent. Subsequent treatments were either spatially selective sprays with glyphosate, to spare any recruiting native rainforest seedlings, or more generalized sprays of grass-selective herbicide. Concentrations and application methods were those typically used by experienced restoration practitioners during establishment and maintenance of rainforest replanting projects in the region. Decisions about the type and delivery of herbicide (e.g., application timing; broad-spectrum vs. grass-selective chemicals; broadcast or localized delivery; use of vehicle-based high-pressure spray or backpack) were made independently and progressively for each site, depending on local topography, how the vegetation had recently developed, and the type of herbicide involved. These decisions were also guided by the underlying goal of longer term cost efficiency within a management context. Overall, during 20 months there were 7–10 herbicide treatments (varying in different parts of the plot) at CloudlandE, and seven at CloudlandW; and during the 9 months at Ringtail there were 6–7 sprays. Some treatments involved broadscale grass suppression, while others were more spatially localized, and targeted specific clumps of herbs, grasses, shrubs or scrambling vines. The nature of these treatments requires close tailoring of actions to suit each local combination of climate and ground-cover species.

Each bird attracting structure (henceforth a "perch") was constructed from an existing multi-branched *Alphitonia petriei* (a common early successional tree), cut at the base, with branches pruned back to a standard form (resulting height about 3–4 m, diameter 5–10 cm with 3–5 branches, embedded in the ground to a depth of 0.5 m). At the base of each sapling there was a water tray (surface 38 × 25 cm, depth 15 cm) which filled mainly from ambient rainfall with occasional hand supplementation, together with two logs (each about 1.0 m long and 20–25 cm diameter). Nine perches were installed in each Works plot in a regular grid of 20 m spacing, with three rows of three perches at each of 20, 40, and 60 m away from, and parallel to, the forest edge.

The pre-existing trees and shrubs in each plot were mapped if they were >2.0 m tall. These consisted of two types: (1) small native regrowth trees (henceforth termed "live trees"), and (2) non-native woody shrubs, all of which were treated with herbicide as part of the initial Works plot establishment (henceforth termed "dead clumps"). Live trees were mostly *Alphitonia petriei*, *Acacia celsa*, *Rhodamnia sessiliflora*, and *Xanthophyllum octandrum*. Care was taken during herbicide applications to avoid these trees. Dead clumps were almost all either lantana (*Lantana camara*) or wild tobacco (*Solanum mauritianum*), and were killed by stem treatment with Starane (fluroxypyr). A few small localized patches of very low growing native shrub (e.g., *Rubus queenslandicus*) or other small woody non-native plants were also killed with herbicide treatment during initial plot establishment.

FIELD DATA COLLECTION

Here we present data from seedling recruitment surveys conducted 20 months after the Works plots were established at the two Cloudland sites (i.e., in July 2013), and 9 months after the establishment of the Ringtail Works plot. Seedling recruitment was measured in two types of systematic search area: strips and circles. Strips were positioned regularly throughout both the Works and Control plots, and circles were placed around perches, live trees or dead clumps. In both types of search, all recruits of woody-stemmed trees or shrubs were counted and identified to species level if they: (1) were seedlings (i.e., excluding a few non-native shrubs that re-sprouted from rootstock); (2) were >10 cm in height; and (3) belonged to any species (native or non-native) which develops a woody stem that can typically exceed 2.5 cm diameter as individuals grow. The height of each stem was also measured, up to a maximum of 2.0 m (after which diameter was recorded).

Strips were transects 20 m by 2 m, positioned parallel to the forest edge. An arrangement of four strips laid end to end spanned the full width of the plot, and this was repeated at eight distances from the forest edge (5, 15, 25, 35, 45, 55, 65, 75 m), giving a total of 32 search strips in each plot (**Figure S2**). Circles had a radius of 2 m, which was placed around either the main stem of tree life-forms or a marked approximate center point within a multi-stemmed clump (the latter being typical for lantana). The number of circles surveyed in a plot depended on how many live trees, dead clumps and perches were present; in the Works plot at CloudlandE these numbers were 7 live trees, 0 dead clumps and 9 perches; compared with 5, 10 and 9 respectively at CloudlandW, and 11, 3, and 9 respectively at Ringtail.

DATA ANALYSES

Counts of recruited seedlings in strips and circles (see above) were standardized to a density unit of stems per 100 m^2 to account for the difference in sampling area between search types (i.e., 40 m^2 for strips and 12.6 m^2 for circles). To examine the effect of grass suppression, mean recruit densities were compared between Works and Control plots, with strips as replicates but excluding all strips that intersected with live trees or dead shrubs, to remove the potential confounding effect of structure on recruitment (no strips intersected with circles around perches; **Figure S2**). Resulting sample sizes were 25 and 26 strips for Works and Control plots respectively at CloudlandE, 21 and 22 respectively at CloudlandW, and 23 and 6 respectively at Ringtail. To test the effect of structural features when acting in combination with grass suppression, we restricted data analysis to the herbicide-treated Works plots and compared mean recruit densities among four contexts: open areas (i.e., strips that did not intersect perches, live trees or dead shrubs) and circles beneath each of these three types of structure. In each case, separate analyses were conducted for native species collectively and non-native species collectively.

Significance tests of treatment effects were conducted using data from the two Cloudland sites, for which 20 month data were available. Tests were performed using generalized linear models based on a Poisson error structure and log link function, implemented using the glm function in the base "stats" library of R (version 3.0.2; R Core Team, 2013). Each model contained three types of fixed effect: restoration treatment (i.e., grass suppression or types of structure as described above), site (CloudlandE or CloudlandW) and their interaction. We evaluated the significance of each term by comparing full models that included all terms and interactions with reduced models omitting each term in turn, using chi-squared tests on the differences in explained variance (Faraway, 2006). Finally, Spearman's correlations were performed to test for an association between numbers of native seedlings recruited and area-specific estimates of species richness for strip and circle searches.

RESULTS

EXTENT OF SEEDLING RECRUITMENT

A total of 1391 woody plant seedlings were recorded overall from strip ($N = 94$) and circle ($N = 40$) surveys at the two Cloudland sites at 20 months (after excluding strips that intersected with pre-existing trees and shrubs). Of these, 345 were seedlings of 28 native rainforest plant species, the most common being the early successional trees *Homalanthus novoguineensis* (Euphorbiaceae, 54%), *Polyscias murrayi* (Araliaceae, 9%), *Alphitonia petriei* (Rhamnaceae, 5%), *Solanum viridifolium* (Solanaceae, 5%), and *Wikstroemia indica* (Thymelaeaceae, 4%) (**Figure 1**). The most commonly recruited native species varied spatially: *H. novoguineensis* was dominant at CloudlandW (70% of 253 recruits) whereas *A. petriei* dominated CloudlandE (18% of 92). Five non-native species were recorded. However, *S. mauritianum* (Solanaceae) was consistently by far the most common, comprising 99% of 1046 seedlings (**Figure 1**); 98% of 97 at CloudlandE and 99% of 949 at CloudlandW. In the following, we consider native and non-native seedlings separately. This enables the response of the dominant *S. mauritianum* to be effectively isolated in the analyses, allowing the response of other species to particular treatments to also be ascertained.

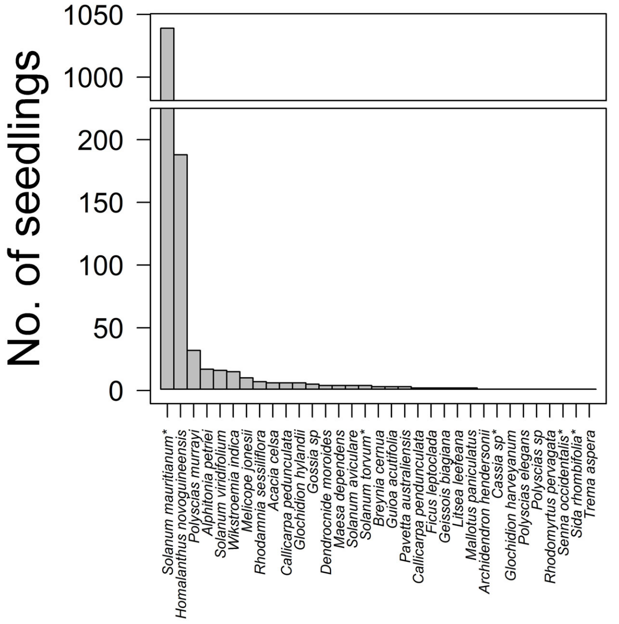

FIGURE 1 | Species frequency distribution for 1391 woody plant seedlings recorded from treated and untreated areas of disused pasture, 20 months after treatments commenced at two sites (CloudlandE, CloudlandW). Total sampling effort covered 4262 m^2, in 94 strip and 40 circle surveys distributed across 2.56 ha. Stars indicate non-native species.

EFFECTS OF GRASS SUPPRESSION ON RECRUITMENT

Site and grass suppression had statistically significant and independent effects on the recruitment of woody seedlings after 20 months, both for native species (GLM site, grass suppression, interaction $\chi^2 = 19.068, 146.987, 1.682$; $P < 0.001, 0.001, 0.195$; **Figure 2A**) and non-native species (GLM site, grass suppression, interaction $\chi^2 = 986.2, 2011.0, 0.0$; $P < 0.001, 0.001, 0.999$; **Figure 2B**). Mean native seedlings recruitment increased marginally following grass suppression with slightly greater recruitment overall at CloudlandW than CloudlandE (from 0 to 1.7 seedlings per 100 m^2 at CloudlandE; from 0.1 to 3.7 seedlings per 100 m^2 at CloudlandW). Recruitment attributable to non-native species was also greater following grass suppression and at the CloudlandW site where mean abundance of stems reached 57.5 seedlings per 100 m^2 (**Table 1**).

EFFECTS OF STRUCTURE ON RECRUITMENT

In areas where grasses had been suppressed, native seedling recruitment at 20 months was further affected by an interaction between site and habitat structures (GLM site, structure, interaction $\chi^2 = 496.61, 2716.65, 164.77$; $P < 0.001, 0.001, 0.001$; **Figure 3A**). At CloudlandE, mean seedling recruitment was 8.8 and 38.8 times greater under perches and live trees respectively, than in open areas were only grasses had been suppressed (**Table 1**). At CloudlandW, the effect of perches was greater and live trees less, engendering a similar response in seedling recruitment to the two structure types (17.4 and 18.1 times greater for perches and live trees, respectively, relative to open areas; **Table 1**). The presence of a third structure type, dead clumps, at CloudlandW induced a larger recruitment response than either perches or live trees (22.9 times more than open areas; **Table 1**).

The response of non-native seedlings was governed by a different form of interaction between site and structure (GLM site, structure, interaction $\chi^2 = 4345.5, 1294.4, 51.7$; $P < 0.001, 0.001, 0.001$; **Figure 3B**). At CloudlandE, mean recruitment of non-native seedlings was marginally less under perches (0.7 times) than in open areas but was enhanced by the presence of live trees (1.7 times greater; **Table 1**). The effect of perches and live trees was both positive and larger at CloudlandW, where seedling recruitment was 1.8 and 3.5 times greater under the two structure types respectively than in open areas. The effect of dead clumps was similar to that of live trees, with 3.1 times more recruitment than in open areas (**Table 1**), although the result for live trees was highly variable (**Figure 3B**).

Overall, the presence of structure also had a localized effect on the proportional representation between native and non-native seedlings. At CloudlandE, abundance of native seedlings was consistently greater than non-native seedlings under all structure types despite the opposite pattern in open areas where only grasses had been suppressed (**Table 1**). At CloudlandW, structure also increased proportional representation of native seedlings though non-native seedlings still remained more abundant (**Table 1**). The 9 month data for the Cloudland sites exhibited similar patterns in response to structure as did the 20 month data though the differences were less pronounced (data not

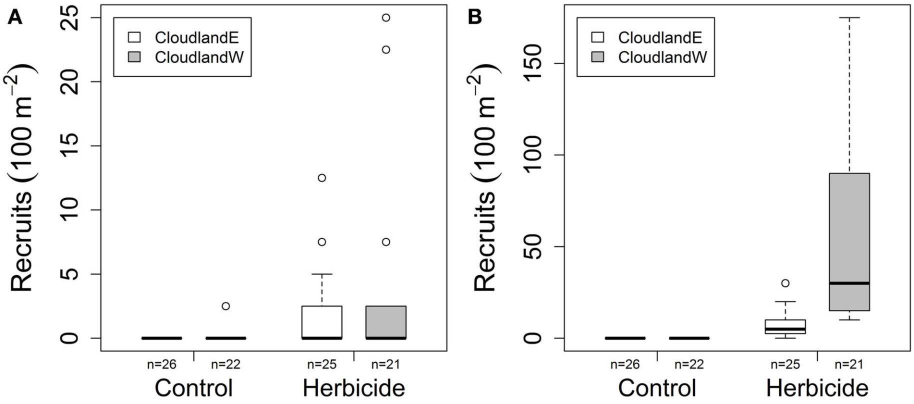

FIGURE 2 | Effect of herbicide treatment on the density of recruited woody seedlings (>10 cm tall) after 20 months at two disused pasture sites (CloudlandE, CloudlandW): (A) native and (B) non-native species. Boxes encompass the upper and lower quartile of data with median value indicated by horizontal line. Whiskers are maximum or minimum values excluding outliers (circles) that are more than 1.5 times the upper or lower quartile.

Table 1 | Effect of herbicide treatment and habitat structures on the density of recruited native and non-native seedlings at three disused pasture sites.

Site	Treatment	Duration (months)	*n**	Search type**	Mean native seedling abundance (per 100 m^2)	Mean non-native seedling abundance (per 100 m^2)
CloudlandE	Control	20	26	S	0.0	0.0
	Herbicide—Open	20	25	S	1.7	7.9
	Herbicide—Perch	20	9	C	15.0	5.3
	Herbicide—Live tree	20	7	C	66.0	13.6
CloudlandW	Control	20	22	S	0.1	0.0
	Herbicide—Open	20	21	S	3.7	57.5
	Herbicide—Perch	20	9	C	64.6	101.7
	Herbicide—Dead clump	20	10	C	84.4	177.6
	Herbicide—Live tree	20	5	C	66.9	203.8
Ringtail	Control	9	6	S	0.0	0.8
	Herbicide—Open	9	23	S	1.1	20.0
	Herbicide—Perch	9	9	C	0.9	13.3
	Herbicide—Dead clump	9	3	C	0.0	0.0
	Herbicide—Live tree	9	11	C	6.5	0.7

**n = number of strip or circle searches; **S = strip search of area 40 m^2; C = circle search of area 12.6 m^2.*

shown). The third, younger, site (Ringtail, surveyed 9 months after establishment), showed a broadly similar pattern: herbicide alone caused a small increase in native and a larger increase in non-native recruitment, whereas live trees were associated with the largest increases in native recruitment (Ringtail site; **Table 1**).

Across both Cloudland Works plots, the mean height of native seedlings recorded in the analyzed strips and circles after 20 months was 61 cm (range 10–250 cm, median = 23 cm, with quartiles at 12 cm and 83 cm, $N = 338$; noting that minimum measurement threshold was 10 cm and individuals >2 m were assigned a height of 2.5 m).

For native recruits, the area-specific species richness values were strongly and positively correlated with their measured densities: for strips in open areas of Control and Works plots Spearman's $r = 0.99$ at CloudlandE and 0.99 at CloudlandW ($N = 51, 43$; $P < 0.001, 0.001$ respectively); and for circles across both types of plot Spearman's $r = 0.92$ at CloudlandE and 0.66 at CloudlandW ($N = 16, 24$; $P < 0.001, 0.001$ respectively).

DISCUSSION

EFFECTS OF BARRIER-LOWERING INTERVENTIONS ON RAINFOREST SEEDLING RECRUITMENT

Almost no new seedlings emerged from the retired pasture Control plots over this study's 20 month period, supporting the idea that recruitment barriers are present. Repeated applications of herbicide to suppress the pasture grasses and herbs had a modest but discernable impact to enhance native seedling recruitment. This finding is broadly consistent with other regional

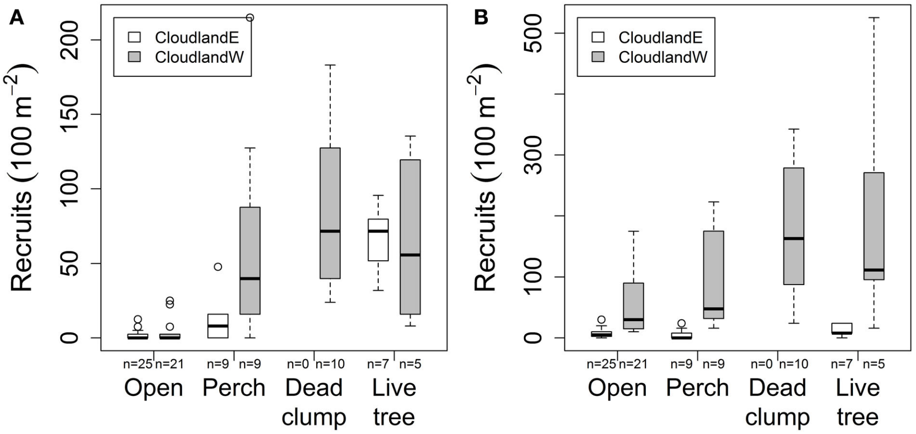

FIGURE 3 | Effect of habitat structures (perches, dead clumps, live trees) on the density of recruited woody seedlings (>10 cm tall) after 20 months at two disused pasture sites (CloudlandE, CloudlandW): (A) native and (B) non-native species. Boxes encompass the upper and lower quartile of data with median value indicated by horizontal line. Whiskers are maximum or minimum values excluding outliers (circles) that are more than 1.5 times the upper or lower quartile.

and international studies where various interventions have been employed to alleviate ground conditions and promote the short term recovery of woody vegetation on degraded tropical land, although many of these did not differentiate between native and non-native species (Shoo and Catterall, 2013).

The recruitment of native rainforest seedlings in pasture where grasses had been suppressed was further enhanced by the presence of structural features, being greater under perches, live trees, and dead shrubs than in open areas. This is consistent with the notion that tropical forest trees can be both dispersal and establishment limited (Holl, 2012), and hence our findings add to a growing body of evidence that combined restoration interventions to simultaneously reduce multiple ecological barriers are more likely to yield tangible progress toward forest recovery than do individual techniques that target single ecological barriers (Holl et al., 2000; Posada et al., 2000; Ammondt et al., 2013). For example, in non-native grasslands of Hawaii, Brooks et al. (2009) found that broadcasting seed in conjunction with grass suppression (by herbicide application) was much more effective in enhancing the woody seedling recruitment than the summed effects of the individual techniques taken separately. Furthermore, strong correlations between species richness and abundance of natives reveal that the response of native rainforest seedlings is not just the result of one or two species but rather is underpinned by a suite of colonizing species.

Previous studies have demonstrated that perches can elevate seed rain into pasture but that this does not necessarily lead to increased seedling establishment (Holl, 1998; Shiels and Walker, 2003; Graham and Page, 2012). Our contrasting result likely stems from the fact that perches were installed in conjunction with active intervention to suppress competition from pasture grasses. However, the extent to which perches enhanced recruitment was modest when compared with the much greater recruitment beneath live trees or dead non-native shrubs. The importance of isolated trees as focal points for bird activity and seedling recruitment in pasture landscapes is well recognized (Toh et al., 1999; Manning et al., 2006). In our study, the larger effect of all trees and shrubs relative to perches is logical for two reasons. First, they had been established for a longer prior time-period, during which repeat frugivore visits could promote greater accumulation of deposited seeds. Second, the trees and shrubs had greater physical volume and architectural complexity, and potentially offered more food or shelter to attract visits by seed-dispersing birds. The dead non-native shrubs were present as a decaying skeletal framework during the 20 months of the study, and live native trees could have provided the birds with more food resources (fruit or insects) as well as shade to ameliorate local climate (thereby potentially increasing seedling germination or survival), but these factors appear relatively unimportant because the dead shrubs tended to produce greatest native seedling recruitment. This finding accords with the suggestion by Toh et al. (1999) that the identity or fruiting status of trees matter less to the activity of seed dispersing birds near forest edges than their structure and suitability as perches.

We might also expect a spatial signature in the pattern of seedling recruitment because seed rain typically decreases with increasing distance from the forest edge, with most seed rain occurring closest to the forest edge (Willson and Crome, 1989; Cubiña and Aide, 2001). However, an exploratory analysis of the effect of distance from the forest edge did not reveal a discernible pattern in seedling recruitment, which instead was highly variable and inconsistent among sites. Given this finding, together with the limited sample size for structures, and also because distance from the edge could conceivably be correlated with several factors which may independently affect recruitment in different directions (seed rain, frugivore visits, slope, exposure to forest-associated marsupial herbivores), we did not include it as a factor in the analyses presented here.

RESPONSES AND ROLES OF NON-NATIVE WOODY SPECIES

The most prominent short term outcome of herbicide-induced grass suppression in our study was the dramatic increase in the abundance and dominance of the non-native wild tobacco (*S. mauritianum*) which constituted the vast bulk of woody stems in open areas, and formed an extensive closed canopy 3–4 m tall over substantial parts of one experimental site (CloudlandW) within 20 months (**Figure 4**). Canopy dominance by a small number of light demanding tree species is not unusual in early stage succession (Guariguata and Ostertag, 2001). However, the non-native origin of the dominant species involved in this instance raises additional considerations for ongoing management.

Life history attributes of *S. mauritianum* include copious seed production and well-developed seed dormancy mechanisms, leading to high seedling recruitment from soil-stored seeds, and this species has been noted for its invasion potential (Florentine et al., 2003; Florentine and Westbrooke, 2003; Witkowski and Garner, 2008). Its relatively high soil seed storage explains why the ratio of recruit density in open areas vs. beneath habitat structures was higher for non-native woody recruits than was the case for native rainforest recruits (**Table 1**). Its fruit are fleshy and bird-dispersed, and hence seedling recruitment was nevertheless further enhanced by habitat structures.

This ability to colonise pasture, form a shady canopy, and quickly produce crops of fruit which are attractive to many seed dispersing frugivores (Florentine et al., 2003) may facilitate subsequent forest regeneration by greatly reducing ecological barriers of ground competition and propagule supply, much more rapidly and extensively than any native rainforest species appears capable of achieving. Indeed, Toh et al. (1999) suggested that lower strata trees such as *S. mauritianum* in the Australian subtropics likely perform an important function in providing the primary means by which taller canopy trees first enter former pasture sites. Our study's finding that native rainforest seedling recruitment was significantly enhanced beneath recently killed dead shrubs (among which *S. mauritianum* was well represented) supports the view that this species has ecological properties which facilitate forest succession. It is therefore possible that the new *S. mauritianum* stands which emerged after pasture suppression will provide positive feedback to enhance future seedling recruitment.

However, it is also possible that germination and growth of seedlings from the native seed rain falling into these stands will be suppressed by competitive interaction with the established *S. mauritianum*, necessitating future treatments to remove or thin the canopy in order to release a further rainforest seedling cohort. The notion that *S. mauritianum* may hinder recruitment of tropical rainforest pioneer and climax species stems partly from observations of an apparent absence of recruitment beneath the canopy of mature plants (Florentine et al., 2003). Shade house experiments show that concentrated aqueous leachates of *S. mauritianum* leaves inhibit the germination of lettuce seeds (*Lactuca sativa*) and can impair the early shoot and root growth of some native tropical rainforest trees (Florentine and Westbrooke, 2003). It is unknown whether these effects extrapolate to local field conditions and how they compare with the alleleopathic potential documented for some native pioneers (e.g., *Acacia melanoxylon*; González et al., 1995). In the Australian subtropics, low recruit abundances were reported beneath both *S. mauritianum* and the common native rainforest pioneer *Homalanthus nutans* which it resembles in stature (Toh et al., 1999).

The extensive recruitment of wild tobacco into retired pasture poses an important management dilemma, since it is necessary to weigh its potential benefits in reforestation against any undesirable ecological consequences of its dominance in early successional plant communities. In Australia, where suppression of invasive non-native species is a central aspect of contemporary restoration activities, routine control of *S. mauritianum* has been widely advocated in recent decades (e.g., Ward et al., 2001), although the species is not currently a declared weed under legislation and had been identified in an early ecological study as a transient successional species in forest regeneration (Williams et al., 1969). Clearly, there is a need for further research into the extent and mechanisms of the various competitive relationships that may occur between established trees and recruiting seedlings of both native and non-native woody species in an oldfield context.

More generally, there is an emerging viewpoint that while some non-native species can cause significant environmental problems, their removal may also have unforeseen consequences,

FIGURE 4 | Visual illustrations of different parts of a "Works" plot. Left panel—14 months after initial herbicide application (viewed from outside plot). Here, bird perches can be seen above the emerging canopy of non-native wild tobacco (*Solanum mauritianum*). **Right panel**—17 months after initial herbicide application (viewed from inside plot). Here, the cover of *S. mauritianum* has shaded the ground sufficiently to suppress pasture grasses.

and some may arguably be useful in restoration (D'Antonio and Meyerson, 2002; Reid et al., 2009). For example, in subtropical Australia, mature stands of the invasive tree *Cinnamomum camphora* in retired pasture support a high diversity of frugivorous birds that facilitate recruitment of a diverse suite of rainforest plants (Neilan et al., 2006; Kanowski et al., 2008). Particularly in moist tropical forest landscapes, some fleshy-fruited non-native tree species may thus provide opportunities for cost effective broad scale reforestation on retired agricultural land. In the restoration context, such realizations have prompted calls for non-native species to be judged on a broader understanding of factors such as their potential transience at a site and their roles in changing processes that influence the course of succession, rather than solely on their origins (D'Antonio and Meyerson, 2002; Davis et al., 2011).

Our findings likewise lead to a conclusion that evaluation of non-native pioneers' transience at sites is critical to making informed management decisions. Like many of its congeners, *Solanum mauritianum* is considered to be highly light-demanding and generally only persists and reproduces as long as the tree canopy remains relatively open (Murphy et al., 2008). This is consistent with evidence that the species declines over time during forest succession (Williams et al., 1969) and with declining light levels following closure of canopy gaps (Enright et al., 1993). In this study, many early-recruiting *S. mauritianum* individuals achieved the species' maximum height of about 4 m within less than 2 years. Most of the native species with which it co-recruited typically reach heights of at least 6 m (some considerably more), and so have the potential to overtop it. While species' growth rates vary, some *H. novoguineensis* individuals had grown as tall as, or taller than, the *S. mauritianum* within the 20 months of this study. Longer-term monitoring is needed to determine the capacity and time required for self-organized recruitment and growth of rainforest trees to shade out stands of *S. mauritianum*, and how well this development trajectory corresponds with time preferences for restoration outcomes.

CONCLUSIONS AND IMPLICATIONS

This study illustrates the role of shifting patterns of competitive inhibition, and its interactions with ecological facilitation, during old field succession in retired agricultural land. Initially pasture grasses outcompete rainforest seedlings, but any forest trees that do establish, as well as invasive woody shrubs, can then outcompete the grass. Subsequently there are also bidirectional potential competitive processes between native and invasive woody species. Understanding of the nature of interactions between different plant functional groups, in terms of both life-form and species origin, is fundamental to these processes, and has important practical applications in the imperative to achieve rapid reforestation of large areas of degraded agricultural land in the tropics. Strategic interventions can accelerate forest recovery, if appropriately selected and timed on the basis of this knowledge. Our findings reaffirm the importance of combined intervention approaches that are designed to overcome multiple ecological barriers, in some cases simultaneously and in others sequentially. Our findings also highlight the importance of retaining or actively reinstating "islands" of woody vegetation as regeneration foci in restoration (Zahawi et al., 2013). Beneath habitat structures, especially live trees and dead shrubs, the recruited woody plant community at our study sites shifted substantially toward greater proportional representation of native seedlings. Thus, the presence of structure is likely to have important consequences for the longer term trajectory of community development.

A crucial gap in knowledge is the timeframe required for larger stature native tree species recruited into the seedling community to overtop the dominant non-native woody pasture invaders such as *S. mauritianum* and whether further interventions such as selective clearing or planting will be required to hasten this process. Our incidental field observations also suggest that the potential importance of herbivory by wildlife in mediating the recruitment of recruited seedlings in retired tropical pastures (see also Holl and Quiros-Nietzen, 1999) merits further investigation.

Empirical tests of the effectiveness of strategies other than active tree planting to simulate regeneration of tropical forest on degraded land have increased considerably over the last two decades (Shoo and Catterall, 2013). However, a major limitation to current knowledge is the short timeframes over which different interventions have been evaluated, and our study is no exception. Clearly, ongoing monitoring would be beneficial to reveal the longer-term trajectories of sites and the efficacy of alternative interventions employed to "kickstart" forest regeneration. In particular, such information would be instructive in determining whether the lower initial cost of less intense interventions considered here is offset by a longer and more expensive subsequent maintenance schedule than is typically needed after high density tree planting. Finally, this study has revealed a strong site based signature on rates of restoration progress, which means that experimental restoration projects need good landscape scale replication before the generality of findings can be fully ascertained.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/Journal/10.3389/fpls.2014.00200/abstract

Supporting Information to accompany: Elgar et al. "Overcoming barriers to seedling regeneration during restoration on tropical pasture land and the potential value of woody weeds"

Figure S1 | Location of experimental sites in relation to each other and existing forest. "Works" plots and "Ungrazed control" plots (80 × 80 m) are depicted by shaded and unshaded boxes respectively.

Figure S2 | "Works" plot with layout of seedling search strips and perches.

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Resource-use efficiency explains grassy weed invasion in a low-resource savanna in north Australia

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Comparative studies of plant resource use and ecophysiological traits of invasive and native resident plant species can elucidate mechanisms of invasion success and ecosystem impacts. In the seasonal tropics of north Australia, the alien C_4 perennial grass *Andropogon gayanus* (gamba grass) has transformed diverse, mixed tree-grass savanna ecosystems into dense monocultures. To better understand the mechanisms of invasion, we compared resource acquisition and usage efficiency using leaf-scale ecophysiological and stand-scale growth traits of *A. gayanus* with a co-habiting native C_4 perennial grass *Alloteropsis semialata*. Under wet season conditions, *A. gayanus* had higher rates of stomatal conductance, assimilation, and water use, plus a longer daily assimilation period than the native species *A. semialata.* Growing season length was also ~2 months longer for the invader. Wet season measures of leaf scale water use efficiency (WUE) and light use efficiency (LUE) did not differ between the two species, although photosynthetic nitrogen use efficiency (PNUE) was significantly higher in *A. gayanus*. By May (dry season) the drought avoiding native species *A. semialata* had senesced. In contrast, rates of *A. gayanus* gas exchange was maintained into the dry season, albeit at lower rates that the wet season, but at higher WUE and PNUE, evidence of significant physiological plasticity. High PNUE and leaf ^{15}N isotope values suggested that *A. gayanus* was also capable of preferential uptake of soil ammonium, with utilization occurring into the dry season. High PNUE and fire tolerance in an *N*-limited and highly flammable ecosystem confers a significant competitive advantage over native grass species and a broader niche width. As a result *A. gayanus* is rapidly spreading across north Australia with significant consequences for biodiversity and carbon and retention.

Keywords: alien invasive species, ecophysiology, water use, carbon uptake, weed invasion, trait-based comparisons, stable isotopes, carbon

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Introduction

Alien plant invasions are considered a major threat globally to biodiversity and ecosystem function (Simberloff, 2011; Vilà et al., 2011; Strayer, 2012). Considerable research effort has gone into understanding the mechanisms that drive invasion success in order to direct effective weed management activities (Blumenthal, 2006; Barney and Whitlow, 2008; Catford et al., 2009).

Invasion drivers vary and are mediated or filtered by characteristics of the ecosystem being invaded, which can also differ in space and time (D'Antonio, 1993; Levine et al., 2003; Theoharides and Dukes, 2007). One of the major drivers of successful invasion is resource competition (Levine et al., 2003; Vilà and Weiner, 2004). Successful invaders are typically considered to possess a superior ability to acquire limiting resources (e.g., light, nutrients), and/or allocate resources to different plant parts for improved performance (Goldberg et al., 1999). Generally, high resource environments tend to be more invasible than low-resource environments (Gross et al., 2005; Funk, 2013); native species are considered more likely to have a competitive advantage over alien plants in low-resource environments (Funk, 2013). However, in a major review on this topic, Gioria and Osborne (2014) found few studies that compared resource competition directly and most studies were undertaken in high resource environments. Many studies were also confounded by factors such as comparisons of different life forms or dominant alien versus subordinate native species. The effects on carbon sequestration and water use when species replacement is by another of the same life form will depend largely on individual species attributes and climate and may be difficult to predict (Cavaleri and Sack, 2010).

This study focuses on the mechanisms facilitating the invasion of C_4 *Andropogon gayanus* Kunth. (gamba grass) in Australia's mesic (>900 mm annual rainfall) savannas. Large areas (>200,000 ha) of invasion are occurring across the 'Koolpinyah surface' (Nott, 1995), a regional geomorphological formation that consists of ancient (Late Tertiary), leached, undulating sandy plains of low soil N and low organic carbon (Scott et al., 2009; Smith and Hill, 2011). Savanna ecosystems being invaded can be considered a resource-limited ecosystem due to these low fertility soils coupled with annual drought (6 months per year) and frequent fire (2 in 3 years) (Hutley and Setterfield, 2008). Despite the limiting resources, *A. gayanus* is one of a number of introduced pasture species that have become successful invaders in this region (Cook and Dias, 2006; Setterfield et al., 2013). Some drivers of *A. gayanus* invasion success have been previously demonstrated. For example, *A. gayanus* produces large amounts of seed annually compared to native grasses (Flores et al., 2005; Setterfield et al., 2005), resulting in high propagule pressure typical of successful invaders (Eppstein and Molofsky, 2007; Catford et al., 2009). Seedling establishment occurs in intact savanna but is greatly facilitated by both canopy cover and/or ground layer disturbance (Setterfield et al., 2005). Like many successful invaders, *A. gayanus* alters the abiotic characteristics of invaded sites to enhance its ability to colonize and survive (Catford et al., 2009). In this situation, the dominant fire regime changes as a consequence of the increased *A. gayanus* derived fuel loads and fire intensity (Rossiter et al., 2003; Setterfield et al., 2010) resulting in reduced canopy cover and ground layer vegetation and increased site suitability for establishment of the invader (Rossiter et al., 2003; Setterfield et al., 2005). These drivers contribute to the initial invasion of *A. gayanus* but the rapid establishment and expansion of this species is likely to be due to other mechanisms that allow the alien species to have competitive advantages over the native species in this low-resource environment.

Studies examining invasion by C_4 grass into low-resource environments suggests the importance of understanding ecophysiological differences between the invaders and native species (Chapin et al., 1996; Williams and Baruch, 2000; Daehler, 2003). In South America's neotropical savannas, the higher maximum stomatal conductance, photosynthesis, and transpiration rates of two invasive C_4 grasses compared to the dominant native C_4 grasses were suggested as partially explaining their invasion success (Baruch and Fernandez, 1993; Baruch and Gomez, 1996). Similarly, in Hawaii, the invasion of alien C_4 *Pennisetum setaceum* (Forsk.) Chiov. was partially attributed to high maximum photosynthetic rates compared to the native C_4 *Heteropogon contortus* (Williams and Black, 1994). Despite this competitive advantage, in both of these studies, the native grass was found to have a greater tolerance to soil water deficit and the growth of the alien grass was constrained by water availability (Baruch and Fernandez, 1993; Williams and Black, 1994). This would limit the spatial distribution and growing season of the alien C_4 grasses, providing insights into how to control these species and restore the ecosystem (Funk, 2013). At present it is uncertain what constraints may limit the spread of *A. gayanus* and this study provides further assessment of the likely ecophysiological mechanisms and their importance driving the replacement of a resident native C_4 grass flora by an alien and invasive C_4 grass. We compared 13 ecophysiological and growth traits of the alien *A. gayanus* and native *Alloteropsis semialata* (R. Br.) Hitchc. In particular, we investigated the (1) diurnal and seasonal patterns of leaf gas exchange and stomatal conductance, (2) maximum photosynthesis and transpiration rates under saturating radiation, (3) photosynthetic responses to leaf to air vapour pressure difference (LAVPD), (4) leaf scale efficiencies of light, water and nitrogen use, (5) canopy scale carbon and water fluxes, (6) foliar nitrogen, and (7) foliar C and N isotopes.

Materials and Methods

Study Location

The study was undertaken at Mary River National Park (formerly Wildman Reserve; 12°43′S, 131°49′E), Northern Territory, Australia. The savanna vegetation at the site is dominated by canopy *Eucalyptus miniata* (Cunn. Ex Schauer) and *E. tetrodonta* (F. Muell) with a cover of 40–50% and a canopy height of 15–20 m. This vegetation assemblage occupies approximately 246, 600 km^2 across Australia's savanna region (Fox et al., 2001). The climate is characterized by distinct wet season (October–March) and dry seasons (May–September), the latter of which has high vapor pressure deficits (VPD, 2–5 kPa; Egan and Williams, 1996). Mean annual rainfall at Mary River National Park is 1433 mm and mean annual temperature is 27°C (Commonwealth Bureau of Meteorology). Soil types at Mary River National Park are sandy loam red and gray Kandosols (after Isbell, 1996) that are characterized by low nutrient levels with a soil organic carbon content (<2%) and low nitrogen content in the surface horizons of from 0.01 to 0.11% (Day et al., 1979; Rossiter-Rachor, 2008). These soils are coarse textured and well drained, but with low water holding capacity.

The native grass understorey consists of perennial C_4 grasses such as *Alloteropsis semialata, Heteropogon triticeus, Chrysopogon fallax, Eriachne triseta* Nees ex Steud., and C_4 annual grasses such as *Pseudopogonatherum irritans* (Br.) and *Sorghum* sp. Following the release of a commercial seed supply in 1983, the alien grass *A. gayanus* (cultivar 'Kent') was planted at a range of locations across northern Australia (Oram, 1987) including near Mary River National Park and has since invaded vast areas of the high rainfall savanna (>1000 mm annual rainfall; Petty et al., 2012; Adams and Setterfield, 2013). It is now a dominant feature of the understorey in the northern section of the national park. *A. gayanus* can form dense monospecific sward up to 4 m high with a biomass of 20–30 t ha^{-1} in heavily invaded patches (Rossiter et al., 2003), with a sharp invasion front adjacent to non-invaded savanna (**Figures 1A,B**). Comparisons were undertaken at paired non-invaded, and invaded sites. Invaded sites had a minimum of 70% cover of *A. gayanus* in the understorey, whereas non-invaded sites had no *A. gayanus* and were dominated by *A. semialata*.

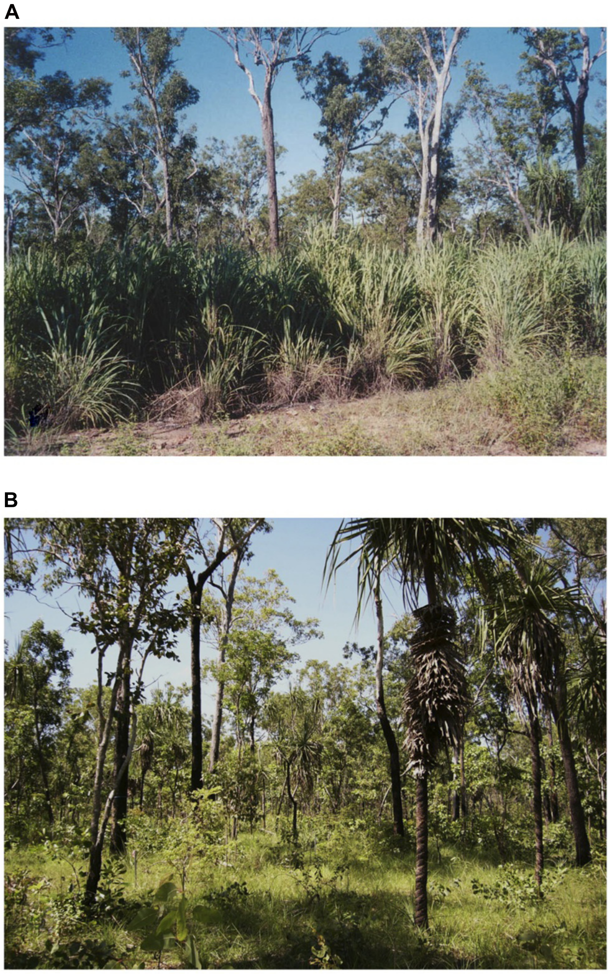

FIGURE 1 | (A) Tropical savanna invaded with *A. gayanus* at Mary River National Park, Northern Territory, Australia. The alien grass forms extensive blocks with a sharp invasion front adjacent to **(B)** uninvaded savanna blocks with an understorey of the native C4 grass *A. semialata*, as used in this study. (Photo credits *N. Rossiter-Rachor*).

Leaf Gas Exchange

Leaf scale physiological traits of *A. gayanus* and *A. semialata* were compared using two approaches. Firstly, observations of diurnal patterns of leaf gas exchange were tracked for the two species using plants from three plots-pairs (*A. gayanus* vs native grasses) within the Mary River National Park. Measurements were made *in situ* during the wet (March) and dry seasons (May) using a portable photosynthesis system (Li-Cor 6400, Li-Cor Inc., Lincoln, NE, USA) on plants within adjacent sward of *A. gayanus* and *A. semialata* across an invasion front (**Figures 1A,B**). Ambient conditions were maintained within the leaf chamber with the instrument in standard measurements mode. Care was taken to ensure the exposure to incident radiation to a leaf was maintained during measurements. Afternoon air temperatures reached 35°C and heating of the instrument occurred requiring regulation of the chamber temperature which was set to 35°C to prevent artificial warming of leaves during measurement.

Native grasses were not measured in the dry season as leaves had senesced by this phase of the seasonal cycle, whereas *A. gayanus* plants still supported green foliage enabling wet and dry season comparisons. Dry season measurements for *A. gayanus* were at the same site using the same population of plants and leaves. These diurnal gas exchange measurements provided *in situ* measurements of leaf performance over a range of leaf and air temperature and light conditions. Secondly, a further set of observations were made during the wet season (March) at an additional three sites within the Mary River National Park. This was undertaken to examine spatial variation of maximum net photosynthesis (A_{max}) and transpiration (T_{max}) of both species under conditions of saturating light. Again, *A. gayanus* and *A. semialata* were sampled across an invasion front at these additional sites.

Gas exchange measurements were made on fully expanded, mature leaves approximately two thirds along the leaf lamina of five randomly selected individual plants. Measurements were made on three leaves per *A. gayanus* plant and two leaves per *A. semialata* plant, given the small plant and leaf size of the latter. This provided a total of 25 leaves sampled across both species per sampling run, which took approximately 1 h to complete. This sampling cycle was repeated continuously from 1000 to 1700 h local time. Variables collected per leaf included leaf temperature (T_{leaf}), leaf to air vapour pressure deficit (LAVPD), photosynthetically-active flux density incident at the leaf surface (PAR), assimilation (A), transpiration (T), and stomatal conductance (g_s).

Gas exchange measurements were made at three additional sites under saturating light conditions with measurements occurring between 11 am and 1500 h local time. These measures were used for the analysis of instantaneous transpiration efficiency (ITE) and intrinsic water use efficiency (IWUE). ITE was calculated as μmol of CO_2 assimilated per mol of water transpired (A/T). IWUE was defined as the ratio of light saturated net assimilation rate to stomatal conductance (A/g_s) which is thought to have low dependence on environmental parameters and reflects intrinsic plant physiological functioning (Jones, 1992). To determine the light compensation points, the light saturation point and apparent quantum yields light use efficiency, (LUE; Kuppers and Schulze, 1985), non-linear regressions ($A = a \times \ln(PAR) - b$) were fitted to the PAR and A data for each species. Instantaneous LUE of each species was quantified as μmol of CO_2 assimilated per μmol^{-1} PAR in the light limited region of the light response curves.

Above-Ground Biomass and Leaf Area Index

In March and May 2003, the above-ground live plant material (leaves and stems) of each species was harvested in three random 2 m × 2 m quadrats at each of the three paired invaded and non-invaded sites used in the gas exchange measurements. Plant material was dried and weighed to give above-ground biomass (AGB) and scaled to leaf area using allometric equations developed for *A. gayanus* (Rossiter, 2001). A generic allometric equation for native grass biomass and leaf area was used as a surrogate for *A. semialata* as this relationship has been shown to hold across a range of northern Australian tropical savanna C_4 grasses (Hutley and Williams, unpublished data). Native grass species used to derive the allometric equations were all C_4 grasses common in these tropical savanna woodlands (Scott et al., 2012) and included *Aristida hygrometrica*, *Chrysopogon latifolius, Sorghum intrans, Heteropogon triticeus, Themeda triandra, Sehima nervosum, Sorghum plumosum, Chrysopogon fallax, Setaria apiculata, Pseudopogonatherum contortum* variously from four sites (Howard Springs, Claravale, Larrimah, and Katherine). Estimates of leaf area sampled from the 2 m × 2 m area enabled the mean leaf area index (LAI) for each site (invaded or non-invaded) to be estimated.

Leaf Nitrogen, Carbon, and Isotopes

All leaves used in the gas exchange measurements of both species were collected, dried, pooled, and ground in a Culatti Type grinder (Model MFC CZ13) with a 1 mm screen. Percent elemental carbon (%C) and nitrogen (%N) and stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios were determined via Dumas combustion in an IsoChrom which was connected to an EA-1110 Elemental CHN-O Analyser. Analysis was conducted by the Australian National University stable isotope facility. Foliar nitrogen values (g N/g leaf) were divided by A_{max} values for each leaf to calculate photosynthetic nitrogen use efficiency (PNUE). Foliar $\delta^{13}C$ values indicate long term water use efficiency (LTWUE, Dawson et al., 2002). Foliar leaf $\delta^{15}N$ values are somewhat indicative of nitrogen source and soil availability, with lower values suggesting preferential uptake of ammonium or higher soil ammonium to nitrate ratios (Handley et al., 1998).

Statistical Analysis

All statistical analyses were performed using SPSS Version 6.0 (2007, SPSS Inc., Chicago, IL, USA). Clear outliers (>2 SD from the mean) were removed prior to analysis. Outliers were clearly identified from raw data plots and data points that were approximately 2 SD from a measurement run mean were examined. In the wet season, the percentage of outliers was larger than 5% and was 11% for *A. gayanus* and 10% for *A. semialata*. None were identified for the *A. gayanus* dry season data set. Variable PAR conditions and shifts in temperature and VPD with cloud cover of the wet season resulted in a population of leaves that were not at a stable equilibrium when measured and were not included in calculations.

Diurnal leaf temperature, PAR, LAVPD, A, T, and gs, at each time period (10:00, 11:00, 13:00, 14:00, and 16:00 or 17:00) were analyzed using a one-way ANOVA to compare differences between the species/season factor (fixed). Data was normal (skewness, kurtosis values <2) and variances homogeneous (Levene's significance test <0.05). Data for diurnal analyses were based on plants at one paired site only. Differences between species and seasons were assessed using the Student-Neumann–Keuls *post hoc* test. The species/season factor included three variables: *A. gayanus* in the wet season, *A. semialata* in the wet season, and *A. gayanus* in the dry season.

Estimates of daily carbon uptake rates and water use per ground area (canopy scale fluxes) were determined by scaling up integrated diurnal measures of A and T, respectively, to rates per square meter ground area using site based LAI estimates. Extrapolation of leaf gas exchange parameters to the canopy scale using LAI is based on the assumption that for these grasses, canopy self-shading is limited and simple scaling using LAI to obtain canopy level estimates is feasible (Larcher, 2003). Leaf scale estimates and foliar $\delta^{13}C$, $\delta^{15}N$, foliar %N, foliar %C, foliar C:N, and PNUE (A_{max}/N per g leaf, PNUE) for each species/season were analyzed using one-way ANOVA and Student-Neumann–Keuls *post hoc* tests to assess differences between species and season (for *A. gayanus* data). Differences in ITE (A/T) and IWUE (A/g_s) for each species/season (all sites data) were analyzed using a one-way ANOVA, Student-Neumann–Keuls *post hoc* tests and pair-wise comparisons with a Bonferroni adjustment.

The LUE of each species was determined as the slope of the linear relationship between PAR and A when light was limiting. Light limitation was assumed to have occurred at PAR $<480\ \mu mol\ m^{-2}\ s^{-1}$. Differences were analyzed using an ANCOVA with PAR as the covariate and species/season as the fixed factor. Differences in A_{max}, T_{max}, and g_s for each species were determined from the light saturated leaves when PAR $>750\ \mu mol\ m^{-2}\ s^{-1}$ for *A. gayanus* and *A. semialata* in the wet season and PAR $>500\ \mu mol\ m^{-2}\ s^{-1}$ for *A. gayanus* in the dry season. Differences were compared using ANCOVA, with PAR as the covariate and species/season as the fixed factor.

Results

Leaf Microclimate

During the wet season, *A. gayanus* and *A. semialata* leaves experienced a broadly similar microclimate in terms of T_{leaf}, leaf incident PAR and LAVPD, enabling direct species comparisons of physiological variables (**Figure 2A**, T, g_s.). Leaf temperatures

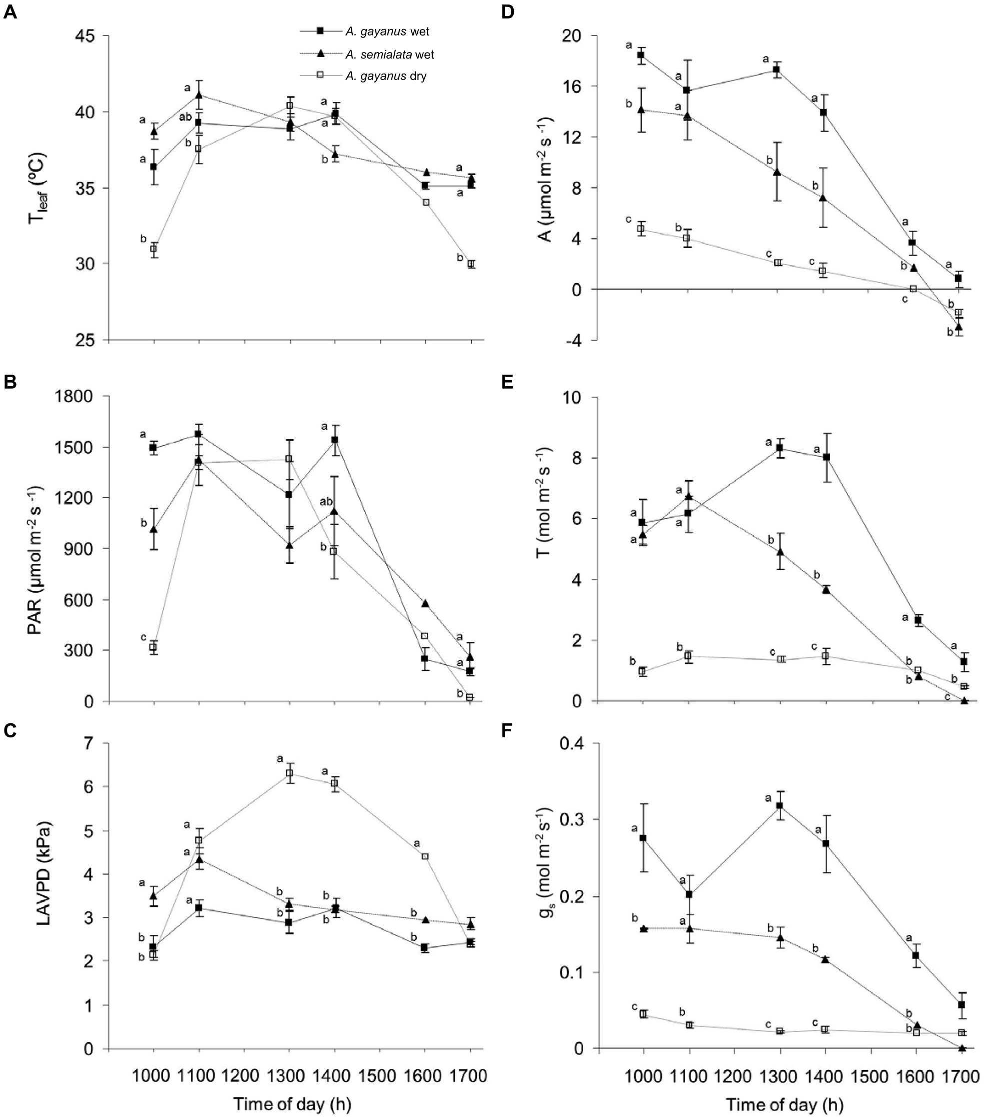

FIGURE 2 | Diurnal patterns of leaf scale (A) temperature (T_{leaf}), (B) photosynthetically active flux density incident at the leaf surface (PAR), (C) leaf to air vapor pressure difference (LAVPD), (D) assimilation (A), (E) transpiration (T) and (F) stomatal conductance (g_s) of *A. gayanus* in the wet (closed squares, $n = 120$), *A. semialata* in the wet (triangles, $n = 120$), and *A. gayanus* in the dry (open squares, $n = 120$) seasons. Error bars represent one standard error of the mean. Different letters at each time indicate significant differences at $P = 0.05$.

of both species were similar throughout the day, although at approximately 1400 h T_{leaf} of *A. gayanus* was higher than that of *A. semialata* (**Figure 2A**). Levels of PAR were also similar for both species except at 1000 h, when PAR was significantly higher for *A. gayanus* (**Figure 2B**). The diurnal range of LAVPD of *A. semialata* leaves was similar to that of *A. gayanus* from 1100 to 1600 h, although leaves of *A. gayanus* had significantly higher LAVPD in the morning and afternoon (**Figure 2C**). By the dry season, early morning, and late afternoon T_{leaf} and PAR were significantly lower for the persistent *A. gayanus* compared to measurements in the wet season (**Figures 2A,B**). The LAVPD of *A gayanus* in the dry season was significantly higher than wet season measurements from 1100 to 1600 h (**Figure 2C**).

Leaf and Canopy Scale Physiology

Both species showed similar decreasing linear trends in A throughout the day; however, in the wet season *A. gayanus* assimilated carbon at significantly higher rates than *A. semialata* (**Figure 2D**). Mean wet season rates of *A. gayanus* A_{max} and T_{max} were 30% higher than *A. semialata* (**Table 1**), with *A. gayanus* maintaining a longer daily period of assimilation compared to *A. semialata*. *A. semialata* exhibited net respiration by 1600 h while A remained positive for *A. gayanus* leaves until 1700 h (**Figure 2D**). Although lower than wet season rates, leaves of *A. gayanus*, were still assimilating carbon and transpiring in the dry season (**Figures 2D,E**) whereas *A. semialata* was physiologically dormant. In the wet season,

TABLE 1 | Mean eco-physiological traits of *A. gayanus* and *A. semialata* in the wet season, and *A. gayanus* in the dry season.

Species/season	AGB ($g\ m^{-2}$)	LAI ($m^2\ m^{-2}$)	A_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	T_{max} ($mmol\ m^{-2}\ s^{-1}$)	g_s ($mol\ m^{-2}\ s^{-1}$)	C uptake ($g\ m^2\ d^{-1}$)	E ($L\ m^2\ d^{-1}$)	ITE (A/T)	LUE (A/PAR)	PNUE (N/A)
(a)										
A. gayanus/Wet	183.9^a (11.78)	0.82 (0.04)	18.31 (0.51)	6.35 (0.17)	0.27^a (0.01)	2.68^a (0.08)	2.51^a (0.06)	1.73^a (0.29)	0.017^a (0.002)	0.06^a (0.003)
A. semialata/Wet	44.0^b (1.77)	0.22 (0.01)	11.81^b (0.55)	4.86^b (0.25)	0.16^b (0.05)	0.54^b (0.08)	0.40^b (0.06)	2.37^{ab} (0.09)	0.022^a (0.002)	0.10^b (0.01)
A. gayanus/Dry	405.5^c (58.86)	1.11 (0.06)	3.31^c (0.29)	1.42^c (0.07)	0.03^c (0.002)	0.97^b (0.12)	0.70^c (0.08)	2.53^b (0.22)	0.008^b (0.003)	0.31^c (0.05)
(b)										
A. gayanus/Wet	15	15	94	94	94	3	3	210	18	210
A. semialata/Wet	15	15	59	59	59	3	3	108	8	108
A. gayanus/Dry	15	15	71	71	71	3	3	120	15	120

[a] *Mean (SE) are given for above-ground biomass (AGB), leaf area index (LAI), maximum net photosynthesis (A_{max}), maximum transpiration (T_{max}), stomatal conductance (g_s), carbon uptake (C uptake), evaporation (E), instantaneous transpiration efficiency (ITE), instantaneous light use efficiency (LUE), photosynthetic nitrogen use efficiency (PNUE). Different superscripts in each column indicate significant differences at $P = 0.05$.* [b] *Number of samples used to derive all variables.*

morning levels of T and g_s for *A. gayanus* and *A. semialata* leaves were similar, however, by 1300 h, *A. gayanus* had significantly higher rates than *A. semialata*. *A. gayanus* was still transpiring water at 1700 h by which time *A. semialata* rates of T and g_s was close to zero (**Figures 2E,F**. Wet season gas exchange (*A*, *T*) was largely driven by PAR (**Figures 2B,D,E**).

Leaf gas exchange rates were extrapolated to a canopy level using LAI estimates to provide mean daily A and T per unit ground area. In the wet season, *A. gayanus* stands assimilated ~5 times more C per day and transpired six times more water than *A. semialata* (**Table 1**). Stand scale *A. gayanus* assimilation and water use in the dry season was still double that of the wet season rates of *A. semialata,* although this difference was not significant (**Table 1**).

Foliar N, C, and Isotopic Signatures

There was no significant difference between foliar %N, %C, or $\delta^{13}C$ for leaves of both species and nor between seasons for *A. gayanus* (**Table 2**). *A. semialata* and *A. gayanus* foliar $\delta^{15}N$ values were similar in the wet season but were significantly lower than dry season values for *A. gayanus* (**Table 2**).

Resource Use Efficiency Traits

Andropogon gayanus and *A. semialata* had similar water use efficiency (WUE) according to three different measurements: IWUE (**Figure 3**), LTWUE ($\delta^{13}C$, **Table 2**) and ITE (**Figure 4**; **Table 1**). The PNUE and A_{max}, T_{max}, g_s of *A. gayanus* in the wet season were significantly higher than *A. semialata* (**Table 1**), while there were no differences in LUE (**Figure 5**; **Table 1**). From the wet to dry seasons, *A. gayanus* leaves showed significant increases in IWUE and ITE (**Figure 4**; **Table 1**); however, there was no change in LTWUE ($\delta^{13}C$, **Table 2**). By the dry season, A_{max} of *A. gayanus* leaves had decreased by 82% (**Figure 5**; **Table 1**) relative to wet season rates, while PNUE was significantly higher (**Table 1**). The LUE of *A. gayanus* was significantly lower in the dry season compared to the wet season (**Table 1**; **Figure 5**).

Discussion

Along with land use change and climate change, alien plant invasion is one of the most threatening processes for the maintenance of biodiversity and ecosystem function. Interdisciplinary research is clearly needed combining ecology, eco-physiology, hydrology, and invasion biology to better understand differences between native and invasive alien species that will assist management and restoration of invaded ecosystems (Gioria and Osborne, 2014). Both instantaneous and time-integrated resource use efficiency (RUE) measures are required to assess performance on short-term (seasonal) as well growth cycles and phases of invasion. In this study, we used comparative measures of both instantaneous (A, A_{max}, T, T_{max}, g_s, ITE, LUE) and integrated measures of RUE (PNUE, LTWUE, LAI, biomass) to assess both resource acquisition and resource conservation performance in a highly seasonal environment.

Tropical savanna may represent a strong 'habitat filter' (after Weiher et al., 1998) as species grow and persist in a low N, annual drought affected, high water deficit, high VPD, and fire prone ecosystem. In such an environment, successful invasive species may exhibit similar resource conservation or RUE traits to native species that enable survival, however, this was not the case in this study. Under wet season conditions of high light, moisture, and N availability, rates of stomatal conductance, A and T of the alien species were 30–40% higher than the native species, with assimilation occurring for an additional 2 months of the year supported by a deep (up to 80 cm) and profusely branched, fibrous root mass (Rossiter-Rachor et al., 2009). Growth was maintained into the dry season with *A. gayanus* stand biomass and LAI exceeding that of the wet season (**Table 1**). This finding is consistent with the meta-analysis of Cavaleri and Sack (2010) who found that invaders typically have significantly higher rates of g_s, water use and assimilation, although their analysis included few studies comparing invasive and native grasses.

TABLE 2 | Mean (SE) and ANOVA results for foliar percentage and isotopic nitrogen and carbon (%) of *A. gayanus* ($n = 210$) and *A. semialata* ($n = 108$) in the wet season and *A. gayanus* in dry season ($n = 120$).

Variable	*A. gayanus* wet season	*A. semialata* wet season	*A. gayanus* dry season	ANOVA
% foliar N	1.1. (0.08)^{a}	1.14 (0.18)^{a}	0.99 (0.15)^{a}	$F_{(2,10)} = 3.78$; $P = 0.695$
% foliar C	46.44 (0.10)^{a}	45.46 (0.39)^{a}	45.93 (0.27)^{a}	$F_{(2,10)} = 3.64$; $P = 0.065$
% foliar C: N	42.92 (2.92)^{a}	42.24 (7.53)^{a}	51.00 (7.50)^{b}	$F_{(2,10)} = 11.67$; $P = 0.002$
δ^{15}N	−3.28 (0.38)^{ab}	−2.37 (0.32)^{a}	−4.52 (0.44)^{b}	$F_{(2,10)} = 6.24$; $P = 0.017$
δ^{13}C (LTWUE)	−11.18 (0.56)^{a}	−11.50 (0.29)^{a}	−12.00 (0.09)^{a}	$F_{(2,10)} = 0.65$; $P = 0.541$

Different superscripts in each column indicate significant differences at P = 0.05.

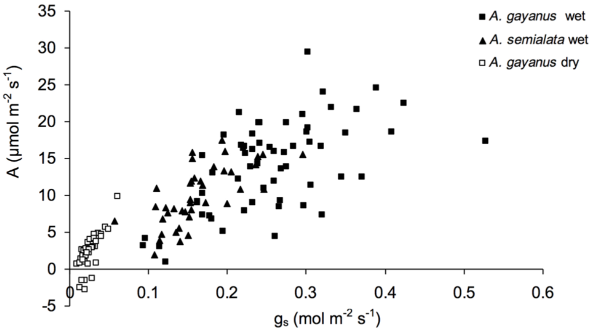

FIGURE 3 | Intrinsic water use efficiency (IWUE) of *A. gayanus* leaves in the wet (solid squares) and dry (open squares) seasons and *A. semialata* in the wet (triangles) seasons.

While rates of A, T, and g_s were higher for the invader, there were no species differences in instantaneous water and light use efficiencies measures (IWUE, ITE, LTWUE, LUE), also consistent with meta-analysis of WUE of Cavaleri and Sack's (2010), even when this analysis was restricted to arid and semi-arid ecosystems (Funk, 2013). While both species are perennial grasses, in this environment leaf function (and age) is essentially annual, with leaf initiation, development, and gas exchange occurring only after the onset of wet season rainfall. This is followed by senescence after seed set in March–April (native species) or May–June (*A. gayanus*). As a consequence, leaves of both species develop in high water availability and low LAVPD conditions with little difference in leaf-scale WUE. *A. gayanus* showed stomatal down-regulation and increases in IWUE and ITE (**Figures 3** and **4**) during the dry season, suggesting physiological plasticity in response to the higher LAVPD and reduced soil moisture availability. Physiological plasticity has been demonstrated for a number of invasive species compared to native species in low-resource environments where resource availability fluctuates (Funk, 2008; Davidson et al., 2011). This is a favorable attribute for persistence in the seasonal tropics, which are characterized by large seasonal changes in resource availability, in particular available N, P, and moisture (Hutley et al., 2000; Soper et al., 2015).

Differences in leaf scale traits alone were unable to explain the 5–10 times greater stand scale biomass accumulation and fourfold increase in LAI of the invader at these sites (**Table 1**). The exception was PNUE, reflecting one of the most significant limiting resource in these mesic savannas, soil available N (Rossiter-Rachor et al., 2009; Soper et al., 2015). Most studies comparing nutrient-use efficiency in native and alien plants have found higher PNUE in the invasive species (Funk, 2013). For example, the alien African lovegrass (*Eragrostis curvula*) had a higher PNUE compared to native grasses in the low-nutrient soils of eastern Australia (Firn et al., 2012). Plant invasion is thought to mostly occur in resource rich environments, with invasion driven by altered growing conditions and release of resources via disturbance that differentially increases an invader's competitive attributes (Daehler, 2003). Recent evidence suggests that invasion and persistence does occur in low resource environments; however, drivers of this are poorly understood (Gioria and Osborne, 2014).

The invasive traits of *A. gayanus* identified in this study exhibit all three attributes suggested by Funk and Vitousek (2007) that are critical for invasion and perseverance in low-resource environments; (1) high resource acquisition and high RUE, (2) an active increase in resource availability following invasion, and (3) continued disturbance following invasion. Firstly, resource acquisition and RUE were exhibited by *A. gayanus* via higher rates of g_s, A, and T, a longer growing season, high biomass and LAI and significantly higher PNUE. Secondly, an invasive species must actively increase resource availability. Comparative values of leaf δ^{15}N (**Table 2**) suggested *A. gayanus* is likely to use more soil ammonium or have increase soil ammonium levels when compared to native grass dominated patches. This is consistent with findings of Rossiter-Rachor et al. (2009) who used labeled ^{15}N experiments that showed *A. gayanus*' preference for ammonium as an N source over nitrate. The presence of *A. gayanus* stimulated soil ammonification and potentially inhibited nitrification (Rossiter-Rachor et al., 2009). This mechanism drives a positive plant–soil feedback that promotes a broader niche width and improved habitat suitability for *A. gayanus*, in this N-limited ecosystem. Thirdly, Funk and Vitousek (2007) suggest an invader must promote continued disturbance that increases resource availability enabling persistence. A feature of *A. gayanus* invasion is high biomass (fuel) production and a shift to a high severity fire regime as described by Rossiter et al. (2003) and Setterfield et al. (2010). Severe invasion reduces woody cover by up to 80% within a decade post invasion (Brooks et al., 2010)

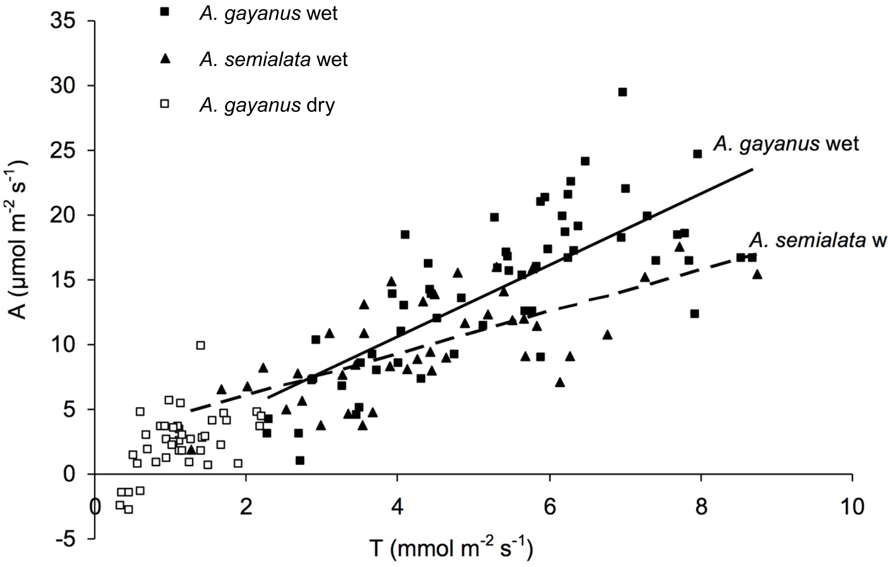

FIGURE 4 | Instantaneous transpiration efficiency (ITE) of *A. gayanus* leaves in the wet (solid squares and solid lines, $n = 210$), *A. semialata* in the wet (triangles and broken lines, $n = 120$), and *A. gayanus* in the dry (open squares, $n = 120$) seasons.

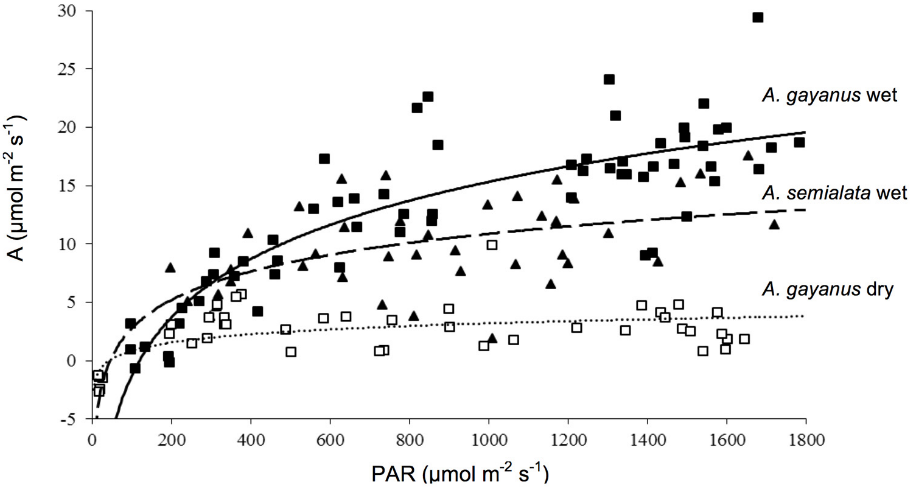

FIGURE 5 | Light response curves for leaves of *A. gayanus* (closed squares, solid line) and *A. semialata* (closed triangles, dashed line) in the wet season and *A. gayanus* in the dry season (open squares, dotted line).

and initiates a grass-fire feedback. The loss in woody cover releases water, nutrient resources and increases radiation to the understory that further accelerates *A. gayanus* growth and invasion.

Conclusion

This study has shown that collectively, instantaneous, and time-integrated RUE traits, invasion-derived feedback loops combined with high propagule pressure confers substantial a competitive advantage to *A. gayanus* over both native grassy and woody lifeforms. These attributes largely explain its current invasiveness and persistence in Australia's low-resource savanna ecosystems. This is an ecosystem transformation that is resulting in a rapid loss of biodiversity and significantly increasing fire risk (Setterfield et al., 2013). This study provides evidence from a seasonal tropical savanna ecosystem to support Funk's (2013) assertion that invasive species in low-resource environments possess traits that allow both increased resource acquisition and resource conservation. This superior capacity of *A. gayanus* to compete for resources also supports modeling predictions of continued rapid invasion across the vast savanna region of northern Australia (Adams et al., 2015).

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Herbaceous plant species invading natural areas tend to have stronger adaptive root foraging than other naturalized species

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Although plastic root-foraging responses are thought to be adaptive, as they may optimize nutrient capture of plants, this has rarely been tested. We investigated whether nutrient-foraging responses are adaptive, and whether they pre-adapt alien species to become natural-area invaders. We grew 12 pairs of congeneric species (i.e., 24 species) native to Europe in heterogeneous and homogeneous nutrient environments, and compared their foraging responses and performance. One species in each pair is a USA natural-area invader, and the other one is not. Within species, individuals with strong foraging responses, measured as plasticity in root diameter and specific root length, had a higher biomass. Among species, the ones with strong foraging responses, measured as plasticity in root length and root biomass, had a higher biomass. Our results therefore suggest that root foraging is an adaptive trait. Invasive species showed significantly stronger root-foraging responses than non-invasive species when measured as root diameter. Biomass accumulation was decreased in the heterogeneous vs. the homogeneous environment. In aboveground, but not belowground and total biomass, this decrease was smaller in invasive than in non-invasive species. Our results show that strong plastic root-foraging responses are adaptive, and suggest that it might aid in pre-adapting species to becoming natural-area invaders.

Keywords: invasion ecology, multi-species comparison, nutrient heterogeneity, phenotypic plasticity, pre-adaptation, root morphology

Introduction

Soil nutrients are generally patchily distributed, frequently at scales as small as a few centimeters (Hodge, 2004). As a result, different parts of a single plant may experience different nutrient conditions. They can respond to this heterogeneity by differentiating their root growth and development between nutrient-poor vs. nutrient-rich soil patches (de Kroon et al., 2009). These plastic root-foraging responses are thought to enable plants to optimize nutrient capture, and increase plant performance (Robinson et al., 1999). Therefore, the capacity for a strong root-foraging response when growing in heterogeneous soil may be expected to be positively correlated to species success or invasiveness. However, it is not known whether or to what extent this is the case.

Many plant species have been introduced to new regions. Some of these species have been able to sustain stable populations (have become naturalized), and some of those have formed new populations and spread rapidly (have become invasive) (Williamson and Fitter, 1996). Most invasions start in anthropogenic environments, and most alien species stay there, but a small subset of alien species manages to also invade natural habitats (Richardson et al., 2000). Frequently, this happens after disturbance events, as predicted by the fluctuating-resources hypothesis (Davis et al., 2000). Few, if any, studies have tested what distinguishes these natural-area invaders from other naturalized alien species. In natural habitats, irrespective of whether they are more or less heterogeneous than anthropogenic environments, alien plants are likely to experience stronger competition from resident species. Under such conditions, it may be especially important to be able to rapidly find and exploit high resource patches (Robinson et al., 1999; Parepa et al., 2013). We therefore expect that successful natural-area invaders have stronger root-foraging responses than unsuccessful ones.

Invasiveness of species is partly determined by their traits (Pyšek and Richardson, 2007; van Kleunen et al., 2010b). Phenotypic plasticity—the change in the expressed phenotype of a genotype as a function of the environment (Bradshaw, 1965)—is frequently mentioned as a trait that potentially promotes invasiveness (Baker, 1965; Richards et al., 2006; Hulme, 2008). Plastic species could express optimal phenotypes under different growing conditions, and this increased environmental tolerance could also allow them to grow in novel environments. In other words, a high plasticity of species in their native ranges could pre-adapt them to the novel environments that they may encounter in their non-native ranges. Some studies found support for a relationship between plasticity and invasiveness (Davidson et al., 2011; Dawson et al., 2012a,b; Keser et al., 2014), but others did not (Schlaepfer et al., 2010; Palacio-López and Gianoli, 2011; van Kleunen et al., 2011). These discrepancies could partly reflect that plasticity of a trait in response to a certain environmental variable is not necessarily adaptive (i.e., does not necessarily increase fitness; van Kleunen and Fischer, 2005). Because plastic changes in root morphology may enhance resource acquisition and thereby performance of the plant, root foraging is likely to be an example of adaptive phenotypic plasticity. Surprisingly, however, it has rarely been tested explicitly whether root foraging increases performance (but see Wang et al., 2013).

To test whether root foraging is adaptive, and whether it generally pre-adapts plant species to invade natural areas, we conducted a multi-species greenhouse experiment. In this experiment, we compared the effect of soil heterogeneity on root morphology and on plant performance of native European plant species differing in their invasion success in natural areas in the USA. We used 24 plant species subdivided into 12 congeneric species pairs from eight plant families, all of which have been introduced to and naturalized in North America. Within each pair, one species is listed as a natural-area invader and the other is not. We grew all species in homogeneous and heterogeneous nutrient environments, and assessed (1) the morphological root-foraging response (root length, root diameter, root biomass, and specific root length) of plants growing in a heterogeneous environment with nutrient-rich and nutrient-poor patches, and (2) the effect of nutrient heterogeneity on the production of aboveground, belowground and total biomass (as a proxy for plant performance) of the plants.

We addressed the following three questions: Are plastic root-foraging responses adaptive in heterogeneous soils? Are European herbaceous plant species pre-adapted to invade natural areas in North America through strong root-foraging responses in heterogeneous soils? And do plant species that are invasive in natural areas experience a more positive or a less negative effect of soil heterogeneity on plant performance compared to species that do not invade natural areas?

Material and Methods

Species Selection and Pre-cultivation

We selected a total of 12 congeneric pairs of herbaceous species from eight plant families. All 24 species are native to Europe, and have become naturalized in North America (Online Appendix I). In this experiment, we were interested in the distinction between plant species that do or do not manage to invade natural areas in the USA. To decide whether the species are natural-area invaders or not, we used The Invasive Plant Atlas of the United States (www.invasiveplantatlas.org), which is a comprehensive compilation of alien plant species that invade natural areas in the US. These natural areas do not include agricultural land or other heavily anthropogenic sites. Within each species pair, one species is listed in this atlas as a natural-area invader in at least four USA states, and the other one is not listed as such in any USA state (Online Appendix I). From here on, we refer to the first group as invasive species and to the second group as non-invasive species. It could be that some of our non-invasive species are no natural-area invaders because they did not disseminate into such habitats yet (van Kleunen et al., 2014b). However, as also our non-invasive species are already widely naturalized in North America (see Online Appendix I), we think that this explanation is quite unlikely.

Because several studies have found a link between relative growth rate and the strength of the foraging response of plant species (see references in Kembel and Cahill, 2005), and others between relative growth rate and invasiveness (Grotkopp and Rejmanek, 2007; Dawson et al., 2011), we reduced this potential confounding factor by balancing the invasive and non-invasive species in each pair with respect to the average plant size. Average height was calculated by averaging the minimum and the maximum height of the species as listed in Rothmaler et al. (2005) (Online Appendix I; size difference between invasive and non-invasive species in the species pairs tested with a paired t-test: $t = 0, p = 1$).

We ordered seeds of our species from botanical gardens throughout Europe and from commercial companies selling wild-collected seeds (see Online Appendix II). Pre-cultivation and the experiment took place in the botanical garden of the University of Konstanz, Germany (N: 47°69′19.56″, E: 9°17′78.42″). For each of the 24 species, we mixed the seeds from the different suppliers. On the 14th of June 2011, we sowed the seeds in trays filled with a 1:1 mixture of sand and fine vermiculite. We first put the trays in a cold room (4°C, 16 h of light per day) for 10 days to break seed dormancy. After that, we put them in a greenhouse compartment and kept them at 70% humidity and temperature between 15 and 25°C. In a preceding pilot experiment, we found that some species germinated faster than others. Therefore, we sowed the seeds of some of the species pairs (genera) 3 days later (see Online Appendix III) to ensure that seedlings would emerge more or less simultaneously.

Experimental Set-up

We filled a total of 358 square 1-L pots with a 1:1 mixture of sand and fine vermiculite. Our experiment lasted for 5 weeks. This timespan enabled us to study early foraging responses as they may be particularly critical for the establishment of plants in nature and also allowed us to harvest the plants before the soil volume was limiting to the extent that we could no longer see foraging responses. To reduce the chances that roots would spill over from one quarter into the others due to limited root space and to prevent minimize nutrient leakage between pot quarters, we placed PVC barriers in the pots to divide them into four quarters (barriers stuck out above the soil, and were pushed all the way down). The central 2 × 2 cm of each pot was left barrier free to allow plants to grow roots in any direction (see **Figure 1**).

After the seedlings had been in the greenhouse for 2 weeks, we transplanted, if available, 16 plants per species into the center of the pots. Because some species germinated poorly, we had <16 plants for *Arctium tomentosum* (8 plants), *Centaurea scabiosa* (6), *Cirsium palustre* (15), *Linaria repens* (12), *Trifolium medium* (1), and *Veronica hederifolia* (9) (see Online Appendix III). The relatively low number of replicates for some of our species could have been problematic, if the objective would have been to get accurate values for each species in our experiment. However, we aimed to get representative values for the invasive and non-invasive species as a group and not as individual species. van Kleunen et al. (2014a) recently showed, using simulations, that the statistical power for detecting differences between groups of species increases with an increasing number of species used, even if the number of replicates per species becomes very low. Pots were placed 7 cm apart on a greenhouse bench, and their positions were fully randomized. At the start of the experiment, we counted the number of true leaves (i.e., excluding the cotyledons), and measured the length and width of the largest leaf on each plant.

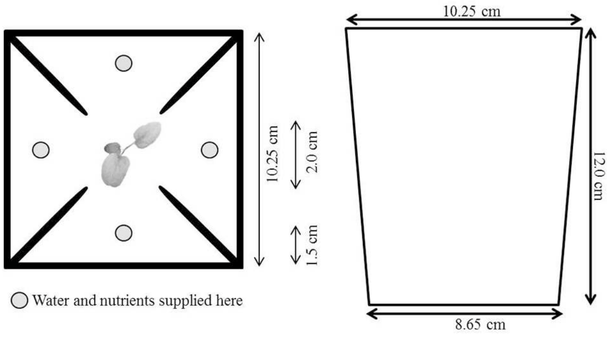

FIGURE 1 | Pot set-up. We filled 1-L square pots with a 1:1 mixture of fine sand and fine vermiculite, and placed PVC barriers in the pots to create four pot quarters. The middle 2 × 2 cm was left open. We supplied plants with a 40ml nutrient solution three times a week with four syringes in the depicted spots (160 ml in total). Plants in the heterogeneous treatment received a high-strength nutrient solution (40 ml 1/2-strength Hoagland solution) in one quarter and a low-strength nutrient solution (40 ml 1/64-strength Hoagland solution) in the other three pot quarters. Plants in the homogeneous treatment received the same total amount of nutrients as in the other treatment but as equal intermediate-strength nutrient solutions (40 ml of ∼1/8-strength Hoagland solution) in all four pot quarters.

Half of the pots of each species were assigned to a heterogeneous nutrient treatment; the other half was assigned to a homogeneous nutrient treatment. When we had an odd number of plants for a species, we maximized the number of replicates for the root-foraging measurements by allocating one plant more to the heterogeneous treatment than to the homogeneous treatment. Plants in the heterogeneous treatment received a high-strength nutrient solution (40 ml of a 1/2-strength Hoagland solution in one pot quarter and a low-strength nutrient solution (40 ml of a 1/64-strength Hoagland solution) in the other three pot quarters. Plants in the homogeneous treatment received the same total amount of nutrients as in the other treatment but as equal intermediate-strength nutrient solutions (40 ml of a c. 1/8-strength Hoagland solution) in all four pot quarters. We watered and fertilized all plants three times per week. Nutrients were supplied 1.5 cm from the pot border through four syringes, which simultaneously dripped the solution into the pot quarters at a rate of 25 ml/min. We tested whether we created a real nutrient gradient in the pots by taking soil samples from pots without plants in the first, third, and the last week of the experiment. We then analyzed the N content of these soil samples (see Online Appendix IV for more details on this procedure). The fertilization regime resulted in a 7.4- (first week of the experiment) to 29-fold (last week of the experiment) difference in N concentration between the high-nutrient and low-nutrient pot quarters in the heterogeneous treatment (Online Appendix IV).

Measurements

Five weeks after the start of the experiment, species were harvested per congeneric species pair (see Online Appendix III for dates). We started with the pairs that had the largest plants. We cut the aboveground biomass at soil level, dried it for at least 72 h at 80°C, and weighed it. We divided the soil of each pot into four parts according to the pot quarters, and washed the roots from the soil. Thick tap roots and large storage roots (lignified roots, thicker than 2 mm) were separated from the other roots, as they contribute relatively little to nutrient uptake. Moreover, although these thick roots were usually in the middle of the pots, they could strongly bias the foraging results if by chance they ended up in one of the pot quarters. We determined the root length and diameter of the roots of the plants from the heterogeneous treatment. In preparation, we stained and preserved the roots from these plants in a neutral-red solution with 0.01% $HgCl_2$ until further analysis. We then determined the length and diameter of all non-storage roots from each pot quarter using a scanner and WinRhizo software (Regent Instruments Inc., Quebec, Canada). Then all roots, also the ones of the plants in the homogeneous treatment, were dried for at least 72 h at 80°C, and weighed. For plants in the heterogeneous treatment, we calculated the specific root length for roots in the high-nutrient pot quarter and the opposing low-nutrient pot quarter as the root length divided by the root biomass.

Statistical Analyses

We analyzed our data with linear mixed models, using the *lme* function (Pinheiro et al., 2010) in the statistical program R (R development core team, 2010). With these models, it is possible to account for the complex nested design of our multi-species experiment by including it in the models as a random structure. These models are also relatively robust when data are unbalanced. Furthermore, the lme function allowed us to correct for heteroscedasticity, caused by the large differences in variance among the species, by adding species variances as a weighting factor (Zuur et al., 2009).

Testing the Adaptive Value of Root Foraging

The average performance of species may increase with their average foraging response, and the performance of an individual plant of a species may increase with its foraging response. As we used multiple species, we could test the adaptive value of plastic foraging responses in the heterogeneous nutrient treatment simultaneously at the among-species level and at the within-species level. We teased apart the effects of the strength of within- and among-species foraging responses on biomass production using a random regression model (e.g., Lane et al., 2012) with within-species mean centering. To do this, we first calculated a foraging index for each individual plant in the heterogeneous nutrient treatment. For root length, root biomass and specific root length, we calculated the index as (the trait value in the high-nutrient patch - the trait value in the opposite low-nutrient patch)/(the trait value in the high-nutrient patch + the trait value in the opposite low-nutrient patch) (e.g., Wang et al., 2013). This way, a high value would indicate a stronger root-foraging response. Because we expected plants to produce thinner roots in the high-nutrient patch, we calculated the foraging index for root diameter as (root diameter in the low-nutrient patch - root diameter in the high-nutrient patch)/(root diameter in the low-nutrient patch + root diameter in the high-nutrient patch). Then we calculated the average of each foraging index per species for each trait, and the deviation of each foraging index of each individual plant from the average foraging index of the species it belongs to. We used linear mixed effects models in which ln(total biomass) of individual plants in the heterogeneous nutrient treatment was used as the response variable, and the average species foraging index (i.e., to test the among-species effect) and the deviations of the individual foraging indices (i.e., to test the within-species effect) as explanatory variables. We included the initial size of the plants as a covariable. To account for variation among species and variation in the within-species effect among species, we included the random effects of species identity and initially allowed the slope of the effects of individual foraging index deviations to vary among species. Because random slopes did not significantly improve model fit in the models with foraging indices of root length, root diameter, and specific root length as explanatory variables, we removed the random slopes from those models.

Testing for Differences between Invasive and Non-invasive Species

For the subset of plants in the heterogeneous nutrient treatment, we tested whether there was a difference in foraging response between invasive and non-invasive plant species. For these analyses, we used as response variables root biomass (biomass of belowground parts excluding large storage structures), root length, root diameter, and specific root length. To quantify the strength of the foraging response, we compared the data from the high-nutrient quarter and the opposite low-nutrient quarter within each pot (i.e., we had two data points per pot). For the whole data set, we tested whether there was a difference in performance between invasive and non-invasive species in response to nutrient heterogeneity. As measures of plant performance, we used total biomass, aboveground biomass, and belowground biomass (all four pot quarters combined). We think that biomass production is a good proxy of performance as size is frequently associated with competitive ability (Dostál, 2011) and seed production (Shipley and Dion, 1992) in herbaceous plants.

For the analyses of foraging responses, the fixed terms of our models included invasiveness of the species (invasive and non-invasive), nutrient patch (high- and low-nutrient patch), their interaction and a measure of initial size of the plants (the length × width of the largest leaf × the number of true leaves). For the analyses of performance traits, the fixed model part included invasiveness of the species, nutrient treatment (homogeneous and heterogeneous treatment), their interaction and initial size of the plants. The hierarchical design of our experiment was included in the models as a nested random term: family/genus/species/pot. In the analyses of performance traits, we had only one value per pot, and accordingly we did not specify "pot" in the random part of these models (i.e., the variance among pots corresponds to the residual variance).

We used likelihood-ratio tests, based on maximum-likelihood estimation, to test which fixed factors were significant (Zuur et al., 2009). We first tested significance of the two-way interaction by removing it from the model and comparing this model to the full model. We then tested significance of the main effects by removing each one in turn and comparing these models to the full additive model (i.e., the model without the two-way interaction). To achieve normality of the residuals, we ln-transformed the data of root length, root biomass, belowground biomass, specific root length, aboveground biomass, and total biomass. For all analyses in which we used belowground biomass, we excluded the data from three plants (two *T. pratense* and one *R. acris*) because of accidentally mixed up roots.

Results

The Adaptive Value of Root-foraging in Heterogeneous Soils

Within species, some strong individual root-foraging responses had positive effects on biomass production (**Table 1**, **Figure 2**). When the foraging response was measured in terms of root diameter and specific root length, these effects were significant. They were marginally significant when measured in terms of root length and non-significant when measured in terms of root biomass (**Table 1**, **Figure 2**). Among species, species with a stronger average foraging response in terms of root length and root biomass had a significantly higher biomass production than species with a weaker average foraging response (**Table 1**, **Figure 2**).

Root Foraging of Invasive and Non-invasive Species in Heterogeneous Soils

In the heterogeneous-nutrient treatment, plants produced significantly longer and thinner roots, and had more root biomass and a higher specific root length in the high-nutrient patches than in the low-nutrient patches (**Table 2**, **Figure 3**). Overall, average root length, root diameter, root biomass, and specific root length (i.e., root length/root biomass) did not differ significantly between invasive and non-invasive species (**Table 2**, **Figure 3**). However, the root-diameter foraging response was stronger in the invasive than in the non-invasive species (**Figure 3B**), as indicated by a significant invasiveness × nutrient patch interaction for root diameter (**Table 2**).

TABLE 1 | Effects of the strength of the within-species and between-species foraging responses (root length, root diameter, root biomass, specific root length) on the ln-transformed plant biomass.

Foraging trait	Among species		Within species	
	Estimate (± s.e.)	*P*-value	Estimate (± s.e.)	*P*-value
Root length	**2.45 (1.04)**	**0.020**	0.62 (0.32)	0.054
Root diameter	5.64 (7.68)	0.464	**4.39 (1.75)**	**0.013**
Root biomass	**2.01 (0.87)**	**0.023**	−0.20 (0.34)	0.553
Specific root length	−0.93 (2.03)	0.646	**0.79 (0.25)**	**0.002**

Presented are the fixed effects estimates of linear mixed effects models, the associated standard errors and p-values of the among-species and within-species effects. The scaled start size of the plants was used as a model covariable; species identity was used as a random factor. Significant results are in bold. Positive estimates indicate a positive effect of root foraging on performance.

Performance of Invasive and Non-invasive Species in Homogeneous and Heterogeneous Soils

Averages of aboveground, belowground, and total biomass did not differ significantly between invasive and non-invasive species (**Table 3**, **Figure 4C**), most likely because we *a priori* selected congeneric species of similar size. Overall, plants produced more biomass in the homogeneous than in the heterogeneous treatment (**Table 3**, **Figures 4A–C**). However, while the non-invasive species had reduced aboveground biomass in the heterogeneous treatment, the invasive species had not (significant two-way interaction in **Table 3**, **Figure 4A**).

Discussion

Our results suggest that root-foraging responses are adaptive in heterogeneous nutrient environments, because we found significant positive effects of root foraging on biomass production of individual plants within species, and significant positive effects of foraging on biomass production of species (**Table 1**, **Figure 2**). We also found stronger root-diameter foraging responses in invasive compared to non-invasive plant species (**Table 2**, **Figure 3B**). In addition, although plants performed worse in

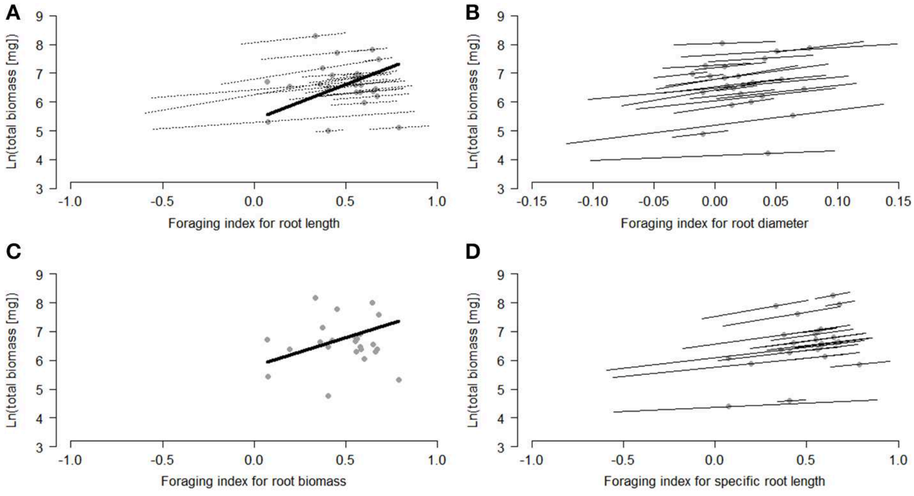

FIGURE 2 | Relationships between root-foraging indices and plant performance [ln (total plant biomass)] within and among the study species. The modeled mean effects of each species are indicated by the dots. When the within-species relationships were significant or marginally significant, they are shown by the thin lines through each dot. When the among-species relationship was significant or marginally significant, it is shown by the thick line. Foraging responses were measured as **(A)** root length, **(B)** root diameter, **(C)** root biomass, and **(D)** specific root length, and performance as the ln-transformed total plant biomass. For calculation of the foraging index see the section ' Testing the adaptive value of root foraging Significant effects are presented as follows: within-species: $p > 0.1$ (no lines), $0.05 < p > 0.1$ (dotted lines), $p < 0.05$ (solid lines); among-species: $p > 0.1$ (no line) and $0.1 < p > 0.05$ (dotted line). The length of the line represents the within-species and among-species spread in the foraging index.

the heterogeneous than in the homogeneous nutrient environment overall, invasive species were better able than non-invasive species of maintaining a relatively high aboveground biomass production in the heterogeneous nutrient treatment (**Table 3**, **Figures 4A–C**). Together, these findings suggest that root foraging is adaptive, and might aid species in becoming invasive in natural areas elsewhere.

Although it is frequently implicitly assumed that phenotypic plasticity of a trait in response to an environmental cue is adaptive, there are still surprisingly few plant traits and environmental factors for which this has been empirically tested (van Kleunen and Fischer, 2005). Well-studied examples are shade-avoidance plasticity, such as stem elongation (Dudley and Schmitt, 1996; van Kleunen and Fischer, 2001), and induced resistance against herbivores (Agrawal, 1998; Agrawal et al., 2002; Crispo, 2007). Despite the large number of studies on plastic root foraging (e.g., Robinson et al., 1999; Kembel et al., 2008), we found only one other experimental study in which its adaptive value was explicitly tested. Wang et al. (2013) found that, in a high-contrast nutrient environment, genotypes of *Potentilla reptans* with a stronger foraging response, measured in terms of root biomass allocation, had a higher total biomass than genotypes with a weaker response. Here, we showed that this holds across a much larger number of herbaceous species.

TABLE 2 | Effects of high- and low-nutrient patches of the heterogeneous treatment on the biomass and morphology of roots of invasive and non-invasive plant species.

Response variable	Initial size	Invasiveness (I)	Nutrient patch (P)	I*P
Root length	**7.51 (0.006)**	0.02 (0.894)	**272.99 (0.000)**	1.10 (0.294)
Root diameter	1.15 (0.283)	0.42 (0.515)	**4.09 (0.043)**	**21.86 (0.000)**
Root biomass	0.78 (0.376)	0.07 (0.797)	**180.40 (0.000)**	1.22 (0.268)
Specific root length	**3.86 (0.049)**	0.54 (0.463)	**43.42 (0.000)**	0.03 (0.865)

Presented are the results of likelihood-ratio tests [log-likelihood ratio and corresponding p-value (in parentheses)], which we used to test whether invasiveness of species, high- or low-nutrient patches and their interaction significantly contributed to the fit of linear mixed effects models. Significant results are in bold.

The strength of the foraging response and its effects on performance can vary with the strength of the nutrient contrast (Wijesinghe and Hutchings, 1999; Wang et al., 2013), and the persistence of nutrient patches in time (e.g., Mommer et al., 2011) and space (e.g., Fransen et al., 1999). If these patch dynamics in nature are faster than the root-foraging responses of the plants, the latter could potentially result in maladaptation (Stuefer, 1996; Dewitt, 1998). However, as long as the foraging response results in at least a temporarily higher nutrient acquisition, it could provide a competitive advantage, and result in a longer term benefit. Another strategy that could allow alien plants to take advantage of resource pulses is to rapidly germinate after a resource pulse. Indeed it has been reported that successful alien species frequently germinate faster than less successful alien species (van Kleunen and Johnson, 2007; Schlaepfer et al., 2010). Recently, Wilsey et al. (2015) showed that invasive species can take advantage of such priority effects. Future studies should address whether invasive species are in general better in taking advantage of resource pulses in dynamic environments, and which strategies they use.

We found that the invasive species showed a more plastic root-diameter foraging response than the non-invasive species (**Table 2**, **Figure 3B**). Similarly, Keser et al. (2014) recently found

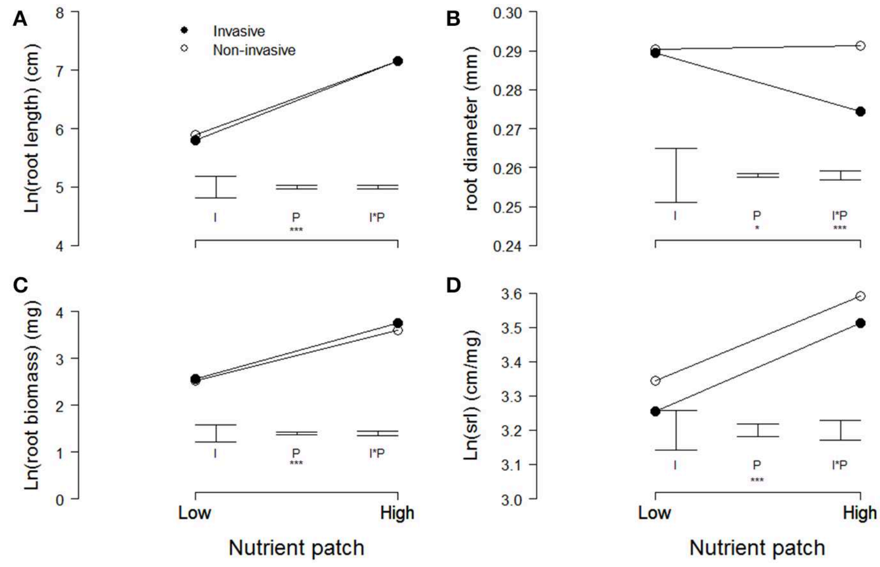

FIGURE 3 | Modeled means of (A) root length, (B) root diameter, (C) root biomass, and (D) the specific root length of invasive (filled symbols) and non-invasive (open symbols) plant species in the high-nutrient pot quarter and the opposing low-nutrient quarter. Please note the cut y-axes. Error bars indicate the modeled standard errors for the effects of invasiveness (I), the nutrient patch (P), and the interaction between the two (I*P). Significant effects are presented as follows: $0.05 < p < 0.1$ (.), $0.01 < p < 0.05$ (*), $0.001 < p < 0.01$ (**), $p < 0.001$ (***).

that invasive clonal plants showed stronger root-biomass foraging responses than non-invasive clonal plants. These results are consistent with the idea that invasive species should have stronger phenotypic plasticity than non-invasive species (Baker, 1965; Richards et al., 2006; Davidson et al., 2011; Palacio-López and Gianoli, 2011). Even though invasive species are expected to be more plastic, many empirical tests did not find support for this (Schlaepfer et al., 2010; Palacio-López and Gianoli, 2011; van Kleunen et al., 2011). Several factors may contribute to the lack of support for this hypothesis in other studies. First, and in contrast to our study, most of the studies did not test whether plasticity in the trait studied was adaptive. Second, many studies did not use genetically identical plant material in the different treatments, and thus may have confounded phenotypic plasticity with genetic differences, if there was genotype-by-environment covariation (Schmid, 1992). We avoided this problem by looking at phenotypic plasticity at the within-plant level. Third, not all studies compared invasive alien species to less invasive alien species, but focused on differences between invasive alien and native species (Davidson et al., 2011; Palacio-López and Gianoli, 2011). Although such comparisons might provide insights into why invasive species can displace certain native species, they do not test why some alien species become invasive and others do not (van Kleunen et al., 2010a; Burns et al., 2013).

TABLE 3 | Effects of nutrient heterogeneity on the performance (aboveground biomass, belowground biomass, and total plant biomass) of invasive and non-invasive plant species.

Response variable	Initial size	Invasiveness (I)	Nutrient treatment (T)	I*T
Aboveground biomass	**23.65 (0.000)**	0.00 (0.975)	**4.08 (0.043)**	**5.33 (0.021)**
Belowground biomass	**4.92 (0.027)**	0.28 (0.599)	**91.36 (0.000)**	2.88 (0.090)
Total biomass	**19.27 (0.000)**	0.06 (0.811)	**35.28 (0.000)**	2.35(0.125)

Presented are the results of likelihood-ratio tests [log-likelihood ratio and corresponding p-value (in parentheses)], which we used to test whether invasiveness of species, soil nutrient heterogeneity and their interaction significantly improved the model fit of linear mixed effects models. Significant results are in bold.

One of the assumptions of the benefit of adaptive phenotypic plasticity for invasiveness is that plastic species could express optimal phenotypes under different growing conditions, and are therefore pre-adapted to become invasive (Richards et al., 2006). We therefore expected that the invasive species would be better capable than the non-invasive species of taking advantage of the high nutrient patches, and thus of maintaining a relatively high performance in the heterogeneous nutrient environment. Indeed, we found this for aboveground biomass, and this suggests that plasticity may aid invasive species to grow better under less favorable heterogeneous growing conditions. However, we did not find significant advantages in terms of belowground and total biomass. Possibly, the experiment did not last long enough for such advantages to become apparent. Furthermore, it has been reported that root foraging may increase competitive potential (Robinson et al., 1999). Therefore, future studies should test whether foraging under competitive conditions increases the performance of invasive species over non-invasive species.

Many other plant characteristics that have been reported to be positively correlated with species invasiveness are also related to nutrient responses. Dostál et al. (2013) found that Central European plant species from more productive habitats and species with a wider habitat-productivity niche in their native range have higher success as alien plant species elsewhere in the world. Dawson et al. (2012a) found that invasive species, just like common native species, can capitalize

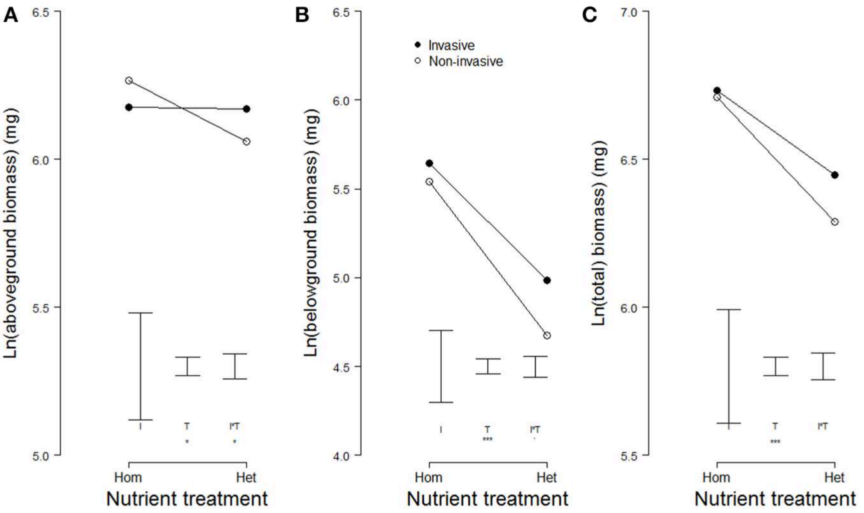

FIGURE 4 | Modeled means of (A) aboveground biomass, (B) belowground biomass, and (C) total biomass of invasive (filled symbols) and non-invasive (open symbols) plant species growing in pots with homogeneously or heterogeneously distributed soil nutrients. Please note the cut y-axes. Error bars indicate the modeled standard errors for the effects of invasiveness (I), the nutrient treatment (T), and the interaction between the two (I*T). Significant effects are presented as follows: $0.05 < p < 0.1$ (.), $0.01 < p < 0.05$ (*), $0.001 < p < 0.01$ (**), $p < 0.001$ (***).

more strongly on extra nutrients than non-invasive alien species, and rare native species, do. Similarly, Dawson et al. (2012b) found that alien plant species with a wider global distribution are better able to capitalize on increased resource availability. Furthermore, Funk and Vitousek (2007) found that in nutrient-poor environments, the nutrient-uptake efficiency of invasive species was higher than that of native species. The ability to effectively exploit soil nutrients may therefore be one of the important factors determining invasiveness of plant species.

Natural areas can be competitive environments, and this may hamper colonization by new species. Strong root foraging could aid colonizing species by allowing them to exploit the available nutrients, and this has been reported to increase the competitive potential of plant species (Robinson et al., 1999). However, Mommer et al. (2012) found that, although a competitively strong species benefited from foraging under competition, a competitively weaker species had a disadvantage because it placed its roots in the empty soil patches instead of in the nutrient-rich patches. Interestingly, we found that on average natural-area invaders had a stronger foraging response than non-invaders. Invasive plant species have been hypothesized to have a stronger competitive ability than non-invasive species (Baker, 1974), although few studies have tested this explicitly (Dawson et al., 2012a). If invasive species are indeed competitively stronger and have a stronger foraging response, this could mean additive benefits for the invasive species in comparison to non-invasive species.

It has been suggested that the importance of certain plant traits for plant invasiveness may change with the stage of the invasion (Pyšek, 1997; Dietz and Edwards, 2006; Theoharides and Dukes, 2007; Dawson et al., 2009; Pyšek et al., 2009). Here, we focused on a transition that has received little research attention: the transition from being naturalized to invading natural areas. We think that this transition deserves special attention, because species that can invade natural areas are likely to become problematic invaders. Our experiment indicates that the potential for nutrient foraging may contribute to species invasiveness in this transition.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpls.2015.00273/abstract

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