Leaf Morphological Traits and Leaf Nutrient Concentrations of European Beech Across a Water Availability Gradient in Switzerland

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Climate change and the expected increase in frequency of dry summers are likely to affect the growth of important tree species. We investigated relationships between morphological and chemical leaf traits of European beech (*Fagus sylvatica* L.) and environmental factors along a water availability gradient consisting of 12 sites located throughout Switzerland. We found that leaf dry mass and leaf area tended to decrease with increasing long-term mean annual precipitation (MAP) and actual to potential transpiration (AT/PT), two correlated variables that form the water availability gradient. These results contrast with those of several other studies, and might be explained by favorable temperature and humidity conditions during leaf formation in spring at the dry sites. Although the relationship was not linear over the whole gradient, the drier sites were characterized by beech with a lower specific leaf area, along with higher foliar potassium (K) and lower foliar nitrogen (N) concentrations. These patterns likely reflect strategies developed as an adaptation to reduced water availability, but they also result from variation in the availability of nutrients in soil across our sites. In the case of N, there are indications that atmospheric deposition plays a role in foliar concentrations.

Keywords: beech forest, drought stress, gradient analysis, leaf functional trait, tree nutrition

INTRODUCTION

Temperature and precipitation are major factors that determine both the occurrence and growth of plant species in forests (Hansen et al., 2001; Morecroft and Paterson, 2006). These two factors have changed and are expected to change further as the global climate warms in response to increased greenhouse gas emissions (IPCC, 2014). The higher temperatures and reduced summer precipitation expected for Europe (Rowell and Jones, 2006; Christensen et al., 2007; Jacob et al., 2014; Stanturf, 2015) are likely to result in drier soils over longer periods (Bell et al., 2010; Karmakar et al., 2016). In forest ecosystems, productivity is expected to be altered, either directly through reduced water availability (Gholz et al., 1990) or through changes in soil biological activity and its effects on nutrient availability (Kreuzwieser and Gessler, 2010).

As the active interface between the forest ecosystem and the atmosphere, leaves play a major role in the carbon, nutrient and water cycle of forests (Jarvis and Leverenz, 1983). The response of leaf functional traits to varying environmental conditions (e.g., light and nutrient availability) is part of the strategies that enable plant species to occupy different environmental niches (Poorter et al., 2009; Wang et al., 2017). Major functional traits include leaf size, which determines water use efficiency and the amount of light intercepted for photosynthesis (Xu et al., 2009; Whitman and Aarssen, 2010; Zhang et al., 2016), and specific leaf area (SLA), the ratio between leaf area and leaf dry mass (Poorter et al., 2009). SLA has been shown to correlate positively with the relative growth rate of plants (Poorter and Remkes, 1990; Cornelissen et al., 1996). It reflects the potential rate of return on investment in a leaf for intercepting light, as SLA increases with decreasing dry matter allocation to leaf tissue for a given leaf area (Cunningham et al., 1999). SLA (or its inverse value, the leaf mass per area LMA) has been shown to vary across annual temperature and precipitation gradients (Wright et al., 2004; Poorter et al., 2009). For example, as a means of reducing water loss, the leaves of species growing in dry environments are often smaller and thicker than those of species growing under wetter conditions, resulting in a lower SLA.

Reduced water availability or dry conditions can impact other leaf traits such as chemistry. Foliar nutrient concentrations can be particularly affected by reduced soil water availability during drought periods through impaired soil-borne uptake of essential nutrients such as nitrogen (Cunningham et al., 1999; Gessler et al., 2004; Villar-Salvador et al., 2015). Finally, environmental factors such as temperature and rainfall can affect the phenolic compounds in leaves (Monteiro et al., 2006). In particular, drought can result in cell wall tightening through the cross-linking of phenolics and lignin monomers to wall polymers, which improves water transport and the mechanical support of aerial structures (Moore et al., 2008; Cabane et al., 2012).

In Switzerland, near-surface air temperature rose by about 2.0°C (compared to 0.9°C globally) between 1864 and 2017, with the largest increase occurring after 1980 (CH2018, 2018). Heatwaves have also become more frequent, and the frequency of summer droughts is expected to increase (CH2018, 2018). European beech (*Fagus sylvatica* L.) is one of the most abundant broadleaved tree species in Switzerland and elsewhere in Europe, and it plays an important economic and ecological role in sustainable forest management (Hlásny et al., 2014). Owing to its sensitivity to drought (van der Maaten, 2012), beech may be negatively affected in its physiological performance, growth and competitive ability under drier conditions (Peuke et al., 2002).

The goal of this study was to assess the response of foliage properties (nutrient concentrations, morphological traits) of beech trees to changes in water availability and soil properties in 12 forest stands distributed along a precipitation gradient across the main regions of Switzerland. For this purpose, we sampled beech leaves in summer 2017 and assessed how leaf traits were related to stand and environmental factors. We hypothesized that (1) morphological parameters such as leaf size and SLA increase with increasing water availability, while biochemical parameters such as the phenol and lignin concentrations are higher under low availability; and (2) the nutrient status of beech leaves is less favorable at sites with lower water availability. Testing these hypotheses will improve our knowledge of how environmental factors influence plant ecological strategies. This gradient study should further shed light on the extent to which beech trees have adapted to conditions of low water availability, and thus how likely they are to adapt to future climate conditions.

MATERIALS AND METHODS

Site Description
Foliage, soil and climatic data were collected from 12 forest sites located in the main geographical regions of Switzerland (**Figure 1** and **Table 1**). Eight of the sites are part of the Swiss Long-term Forest Ecosystem Research programme (LWF, Schaub et al., 2011) and of the pan-European Level II plot network of ICP Forests[1]. The elevation of the study sites ranged from 470 (Vordemwald) to 1210 m a.s.l. (Isone). Except at Vordemwald, beech was either the main tree species or one of the main tree species at the study sites.

Foliage Data
Sampling in the Field
Five beech trees belonging to the dominant or predominant classes were selected on each plot. One to two sun-exposed branches per tree were cut from the upper third of the crown in mid-August 2017, before the onset of autumnal yellowing (Thimonier et al., 2010; Rautio et al., 2016). The cut branches were transported to the WSL and stored at 2°C until further processing.

Processing in the Laboratory
For each sample tree, 100 leaves were clipped from the branches, put in paper bags, and stored again at 2°C. Subsamples of 30 leaves per tree were weighed then scanned by transparency with a 200 dpi resolution, using a flatbed scanner (Epson Perfection V800, Seiko Epson Corporation, Japan). The acquired images were automatically processed by the software WinSeedle (winSeedle 2006, Régent Instruments, Quebec, QC, Canada) to obtain the area, length and width of individual leaves. The leaves were not rehydrated before scanning, and it is possible that they shrank slightly during processing and storage. However, as all samples were handled in the same way, comparability of the obtained values was ensured.

Immediately after scanning, all 100 leaves were weighed. The dry mass of 100 leaves was then determined after drying at 65°C until a constant weight was reached. SLA was calculated as the leaf area of the 30 scanned leaves divided by their dry mass. As we did not dry separately the 30 leaves that had been scanned, their dry mass was estimated by taking the proportion of dry matter in all 100 leaves and applying it to the mass of 30 leaves as determined just before scanning. A subsample of ca. 4 g of whole leaves (dried at 65°C) was ground with a mixer mill (MM400, Retsch GmbH, Haan, Germany) for chemical analysis.
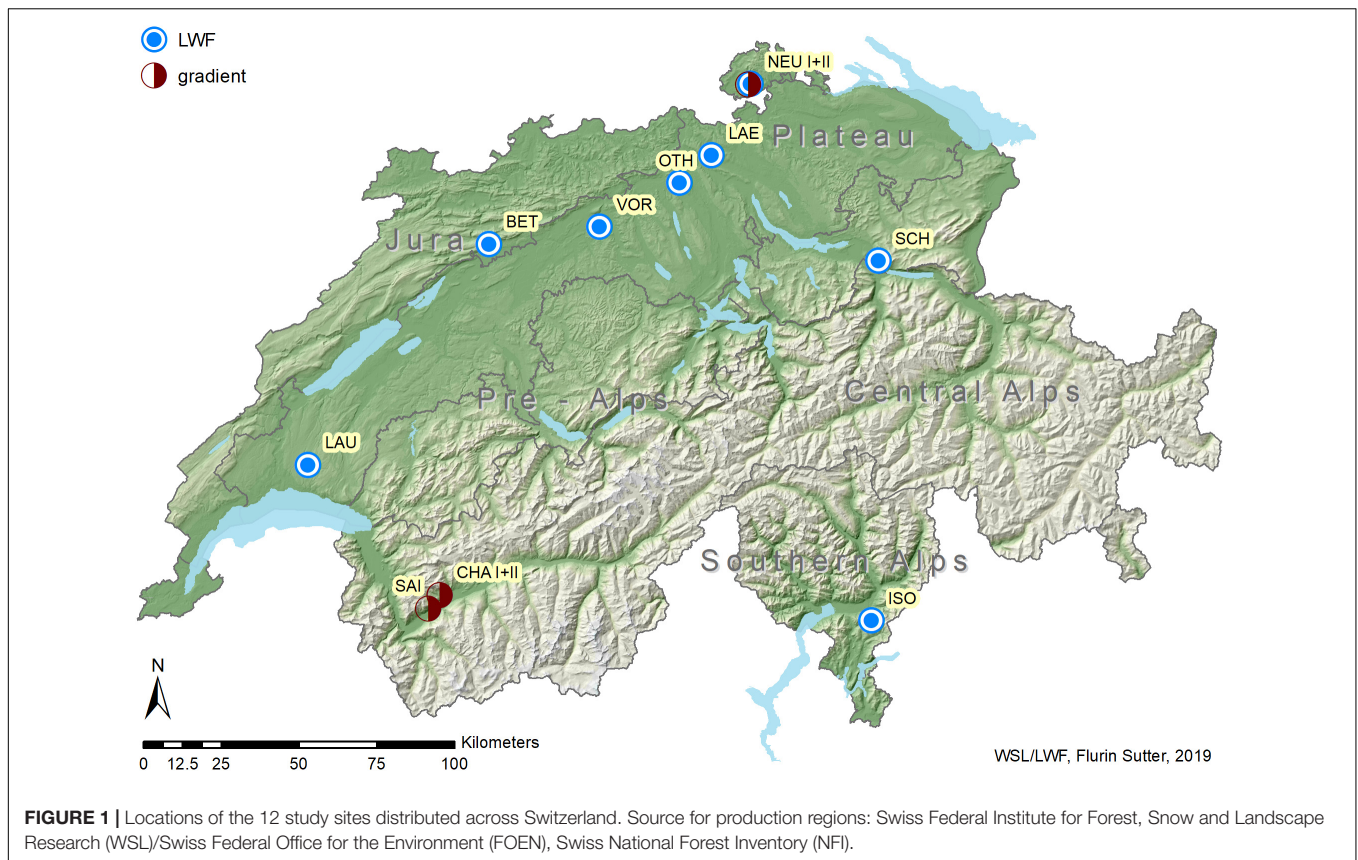
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[1]www.icp-forests.net

FIGURE 1 | Locations of the 12 study sites distributed across Switzerland. Source for production regions: Swiss Federal Institute for Forest, Snow and Landscape Research (WSL)/Swiss Federal Office for the Environment (FOEN), Swiss National Forest Inventory (NFI).

TABLE 1 | Site and tree characteristics of the 12 pure or mixed stands of European beech (*Fagus sylvatica* L.).

Site name	Code	Region	Latitude (N)	Longitude (E)	Elevation (m. a.s.l)	Orientation	Slope (%)	MAT (°C)	MAP (mm)	AT/PT	AWC (mm)	Soil type (FAO, 1988)	DBH (cm)
Bettlachstock	BET	Jura	47°14′	07°25′	1150	S	80	7.1	1685	1	349	Rendzic Leptosol	51 (6)
Chamoson I	CHA I	Western Central Alps	46°12′	07°12′	875	NE	65	8.5	870	0.80	229	Eutric Cambisol	39 (8)
Chamoson II	CHA II	Western Central Alps	46°12′	07°12′	880	S	90	8.6	815	0.77	181	Rendzic Leptosol	18 (9)
Isone	ISO	Southern Alps	46°08′	09°01′	1220	NE	58	6.1	1846	0.97	194	Podzol	46 (7)
Lägeren	LAE	Plateau	47°28′	08°22′	680	S	50	8.2	1148	0.95	307	Dystric Cambisol	70 (20)
Lausanne	LAU	Plateau	46°35′	06°40′	805	NE	7	7.6	1263	0.98	340	Dystric Cambisol	63 (6)
Neunkirch I	NEU I	Jura	47°41′	08°32′	580	N	58	8.8	953	0.80	170	Rendzic Leptosol	54 (14)
Neunkirch II	NEU II	Jura	47°40′	08°31′	530	SW	50	9.1	949	0.69	107	Rendzic Leptosol	45 (8)
Othmarsingen	OTH	Plateau	47°24′	08°14′	485	S	27	9.4	1113	0.95	286	Haplic Acrisol	80 (14)
Saillon	SAI	Western Central Alps	46°10′	07°09′	890	SE	55	9.4	829	0.83	421	Calcaric Regosol	35 (5)
Schänis	SCH	Pre-Alps	47°10′	09°04′	735	W	60	8.1	1834	1	336	Eutric Cambisol	48 (2)
Vordemwald	VOR	Plateau	47°17′	07°53′	480	NW	14	9.1	1153	1	396	Dystric Planosol	45 (14)

MAT, mean annual temperature (1981–2010); MAP, mean annual precipitation (1981–2010); AT/PT, mean actual to potential transpiration in June to August (1981–2010); AWC, plant available water capacity of the soil between 0 and 200 cm depth; DBH, stem diameter at 1.30 m above ground level (mean of the five sampled trees; standard deviation in brackets).

Chemical Analysis

A CN element analyzer (NA 2500, CE Instruments, Wigan, United Kingdom) was used to analyze total carbon (C) and total nitrogen (N). Concentrations of the macronutrients sulfur (S), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg), and the microelements aluminum (Al), boron (B), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), zinc (Zn) and

barium (Ba) were determined by microwave digestion with 5 M nitric acid (HNO_3) and 0.5 M fluoric acid (HF), followed by analysis with ICP-AES (Optima 3000, Perkin-Elmer, Waltham, Massachusetts, United States). All samples were analyzed in duplicate with accredited methods (ISO 17025) by the WSL laboratory, which regularly participates in international ring tests (e.g., Fürst, 2018). The carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope

ratios were measured simultaneously during a combustion process with additional oxygen in an elemental analyzer (EA3000, EuroVector, Milano, Italy) connected to an isotope mass spectrometer (Delta V, Thermo Fisher, Bremen, Germany).

Water extractables were determined by extracting 1 g of milled sample three times with 25 ml of hot ($85 \pm 5°C$) water and once with water at room temperature. Extraction times were 15 min at each step. Phenolic compounds in the water extracts were determined by the Folin-Denis colorimetric method (Swain and Hillis, 1959). Klason lignin was determined by hydrolyzing 0.3 g of pre-extracted (hot water and ethanol) sample with 3 ml of 72% sulfuric acid (1 h at 30°C) and, after addition of 48 ml H_2O, autoclaving for 1 h at 121°C. Soluble lignin was determined by UV absorption (205 nm) of the hydrolyzate (Dence, 1992). Total lignin was calculated as the sum of Klason lignin and soluble lignin (Heim and Frey, 2004).

Environmental Data

Climatic Data

Mean annual temperature (MAT) and precipitation sum (MAP) for the period 1981–2010 were derived specifically for the coordinates of each site, by interpolating weather data from the meteorological network of MeteoSwiss. These data were provided by Remund et al. (2014) (**Table 1**).

To compare the meteorological conditions of 2016 and 2017 with the long-term climatic conditions at the 12 study sites (see "Water availability gradient"), data from the following MeteoSwiss grid-data products were used: interpolated fields of monthly mean temperatures (TabsM), of monthly and annual precipitation sums (RhiresM and RhiresY), of monthly mean temperatures for the norm period of 1981–2010 (TnormM8110), and of mean monthly and annual precipitation sums for the norm period of 1981–2010 (RnormM8110 and RnormY8110). MeteoSwiss calculated these grid data products over the area of Switzerland by interpolating high resolution meteorological station data to a geographical grid (longitude, latitude) with a spacing of ca. 2.2 km. Details about the interpolation methods and the accuracy of the products can be found in MeteoSwiss (2016a,b). For this study the data from the grid points closest to the 12 sites were extracted. The spatial resolution of these climatic data is coarser than that of the site-specific MAP and MAT values provided by Remund et al. (2014), and there are occasionally some substantial differences between the two sources of data. However, deviations of single years from the long-term means are expected to be in the same range.

Soil Data

Soils were described morphologically from soil pits dug down to an average depth of 2.1 m. Criteria such as color, structure, volumetric stone content and the occurrence of redoximorphic features were used to differentiate between pedogenetic horizons (Walthert et al., 2004). For chemical analyses, an average of eight soil samples per soil profile was taken at various depths depending on the depths of the horizons. Organic horizons were also sampled in cases where a forest floor was present. Additionally, to determine the density of the

soil, volumetric samples were collected at selected soil depths using steel cylinders 1000 cm^3 in volume. In stony soils, soil samples were excavated and the hole volume was determined by refilling it with quartz sand. Soil samples for chemical analyses were dried at 40–60°C until reaching a constant weight and sieved to 2 mm. An aliquot of each sample was ground for 3 min using a vibrating ball mill (MM2000, Retsch GmbH, Haan, Germany) with zircon-grinding tools. The volumetric samples for the determination of soil density were dried at 105°C for 48 h.

Plant available water (AWC), i.e., the amount of water between field capacity and permanent wilting point, was calculated according to Teepe et al. (2003) to a depth of 200 cm considering soil texture, soil density, humus and stone contents. The water balance was modeled in daily time steps for the period 1981–2010 to estimate the drought index AT/PT, i.e., the ratio of actual to potential transpiration, with lower AT/PT values indicating more severe water shortage (maximum value = 1). In our study, the drought index AT/PT represents an average long-term yearly summer drought, as it was calculated by dividing the sum of all daily values of AT by the sum of all daily values of PT from June to August 1981–2010. The water balance was modeled with the Coup Model (Jansson and Karlberg, 2011), a coupled heat and mass transfer model for soil–plant–atmosphere systems, after calibrating the model with data from LWF plots. Model performance, as assessed by means of goodness of fit (PBIAS and wR^2) between modeled and measured soil water potential time series was judged to be satisfactory (Walthert et al., 2015; for more details see Walthert and Meier, 2017).

Total P (P_{tot}) and organic P (P_{org}) were analyzed (in triplicate) using the ignition method of Saunders and Williams (1955). A fraction of each soil sample was ignited at 550°C. The ignited residue and an equivalent fraction of unignited soil were then extracted with 0.5 M H_2SO_4. The extracted orthophosphates from the ignited and unignited soil samples, corresponding to P_{tot} and inorganic P (P_{inorg}), respectively, were quantified by colorimetry. The organic fraction P_{org} was calculated as the difference between P_{tot} and P_{inorg}.

Total carbon (C_{tot}) and total nitrogen (N_{tot}) were determined with a CN analyzer NC 2500 (CE Instruments, Italy). The content of organic carbon (C_{org}) of samples with a pH ($CaCl_2$) < 6.0 was assumed to be equal to the content of C_{tot}. Samples with a pH > 6.0 were assumed to potentially contain carbonates and were thus fumigated with HCl vapor prior to the CN analysis (Walthert et al., 2010).

Exchangeable cations were extracted (in triplicate) from the 2-mm-sieved soil in an unbuffered solution of 1 M NH_4Cl. The element concentrations in the extracts were determined by ICP-OES (Optima 3000, Perkin–Elmer, Waltham, Massachusetts, United States). In samples with a pH ($CaCl_2$) < 6.5, the contents of exchangeable protons were calculated as the difference between the total and the Al-induced exchangeable acidity, as determined by the KCl method (Thomas, 1982). In samples with a higher pH, the contents of exchangeable protons were assumed to be negligible. The effective cation-exchange capacity (CEC) was obtained by summing the charge equivalents of exchangeable

Na, K, Mg, Ca, Mn, Al, Fe and H. The base saturation (BS) was calculated as the ratio of the sum of exchangeable Na, K, Mg and Ca to the CEC.

Pools (mol_c ha^{-1}) and mean contents ($mmol_c$ kg^{-1}) of elements were calculated for different depths (0–10, 0–20, 0–40 and 0–80 cm, including organic layers when present) by considering the amount of fine earth in the involved pedogenetic horizons. In a preliminary analysis, we tested all depths for both the pools and mean contents of elements in order to determine which variant provided the best correlations between foliage nutrient concentrations and soil data. However, none of the variants clearly out-performed the others. Therefore, we used only the element contents from 0–80 cm depth in the further analyses, except in the cases of P_{org}, P_{inorg}, P_{tot}, C_{org}, N_{tot} and C/N, for which we used values from 0–10 cm depth, where the organic carbon content and biological activity are highest.

Further Data

Further environmental data used in our analysis included information on the trees and the stand structure, the indicator value of ground vegetation with respect to site conditions, and atmospheric deposition of nitrogen.

The tree stem circumference at 1.30 m above ground level (**Table 1**) was measured with a measuring tape on the day of foliage sampling. The age of the stand was determined by taking increment cores from a few dominant trees in the buffer zone of the sites. The leaf area index (LAI) of the stands was obtained using a regression model developed by Schleppi et al. (2011), using tree height and the coverage of the herb, shrub and tree layers assessed during vegetation surveys (Thimonier et al., 2011) as predictors.

Synthetic indices of the ecological conditions at the sites, based on these same vegetation surveys (Thimonier et al., 2011), were calculated by averaging the Landolt indicator values (Landolt, 1977) of all species recorded in the herb layer. Landolt's values indicate the ecological range of species of the Swiss flora along gradients of soil nutrient (mostly nitrogen) availability (N), soil pH (R), soil moisture (F), light (L), and temperature (T) using an ordinal five-point scale (1 to 5; **Table 2**). Hereafter the ecological values per site are presented with *Landolt* in subscript.

The annual atmospheric deposition of the reduced and oxidized forms of nitrogen (N) was modeled for the coordinates of each site using the emission based model developed for Switzerland at a high spatial resolution (1×1 km^2 grid or finer), run for the period 2013–2017 (Rihm and Achermann, 2016). Both the wet deposition pathway (input via rain, snow or hail) and the dry deposition pathway (deposition of gases and aerosols) were quantified. As variables in our analysis, we selected wet deposition (WD) of ammonium (NH_4^+) and nitrate (NO_3^-) and dry deposition (DD) of ammonia (NH_3), as single factors and in combination, and the total deposition of N (TD). **Table 2** provides an overview of the environmental variables used in this study.

Water Availability Gradient

The water availability gradient was built by ordering the 12 sites either according to the purely climatic factor MAP (1981–2010)

or according to the pedo-climatic drought index AT/PT (ratio of actual transpiration (AT) to potential transpiration (PT) in June to August 1981–2010) (**Table 1**). The precipitation gradient was related to the temperature gradient, with a negative correlation emerging between MAP and MAT (Spearman $r = -0.69$, $p < 0.05$; **Supplementary Figure 1A** and **Supplementary Table 1**). Furthermore, the AT/PT ratio and MAP were positively and significantly correlated ($r = 0.83$, $p < 0.001$; **Supplementary Figure 1B**).

Our water availability gradient is based on long-term climatic or pedo-climatic averages, but foliage was sampled during a particular year only, and foliage traits are likely to be affected by weather conditions in the current year (2017) or the year preceding leaf formation (2016). We therefore evaluated how these 2 years compared with long-term means.

The year 2017 was generally drier, while 2016 was generally wetter than average at our sites (**Supplementary Figure 2A**). Because there were no marked regional differences in precipitation patterns, the water availability gradient was maintained, i.e., the precipitation distribution across sites in 2017 reflected fairly well the long-term water availability gradient. The year 2017 was not only drier but also markedly warmer (by $0.8 \pm 0.2°C$) than average at all sites, notably during the March-August period (**Supplementary Figure 2B**). The year 2016 showed no particular deviations for temperature, but it should be noted that the temperature at the warm and dry sites in Valais (CHA I, CHA II, SAI), was higher by ca. 1°C in 2016 compared to the average.

Data Analysis

We calculated the site averages of leaf nutrient concentrations and leaf morphological traits and used Spearman correlation analyses in SPSS (version 25) to quantify how foliar variables were related to each other and with environmental factors. Foliar nutrient concentrations and ratios were compared to the available reference values indicating the adequate to optimal range of nutrition for beech (Mellert and Göttlein, 2012). We calculated linear regressions between variables by applying a robust regression method (ROBUSTREG procedure in SAS 9.4; SAS Institute Inc., Cary, NC, United States). This method makes the estimation of the slope and intercept less sensitive to extreme values (e.g., Gonsamo et al., 2018).

We further conducted a principal component analysis (PCA) of the data set with individual values for each tree using the *ade4* (Dray and Dufour, 2007) package in R version 3.5.1 (R Core Team, 2015) to extract orthogonal multivariate axes of leaf variables. The PCA was run using 13 variables including all macronutrients, leaf area, SLA and isotopes ratios. Microelements were included as supplementary variables only. We then overlaid the environmental factors (climatic data, elevation, N deposition, Landolt values and soil chemical parameters), also as supplementary variables, on the correlation plots. Further, we built linear regression models using the GLM procedure in SAS 9.4 to explore the influence of the environmental variables on selected leaf traits, by introducing manually (stepwise) up to three explanatory variables out of those listed in **Table 2**.

TABLE 2 | List of environmental variables used in this study.

Category	Variable	Description	Unit
Climate	MAP	Mean annual precipitation (1981–2010)	mm
	MAT	Mean annual temperature (1981—2010)	°C
Pedo-climatic variable			
	AT/PT	Mean ratio between actual (AT) and potential transpiration (PT) in June to August (1981–2010)	–
Site variables			
	Elevation	Elevation above sea level (a.s.l.)	m
	LAI	Leaf area index (modeled)	–
	Circumf	Tree stem circumference at 1.30 m above ground level	mm
	Age	Average age of the trees	–
Soil variables			
	AWC	Plant available water capacity of the soil between 0 and 200 cm depth	mm
	pH	pH ($CaCl_2$) (0–10 cm)	–
	Ca_{soil}	Exchangeable calcium content (0–80 cm)	$mmol_c\ kg^{-1}$
	K_{soil}	Exchangeable potassium content (0–80 cm)	$mmol_c\ kg^{-1}$
	Mg_{soil}	Exchangeable magnesium content (0–80 cm)	$mmol_c\ kg^{-1}$
	Mn_{soil}	Exchangeable manganese content (0–80 cm)	$mmol_c\ kg^{-1}$
	Fe_{soil}	Exchangeable iron content (0–80 cm)	$mmol_c\ kg^{-1}$
	BS	Base saturation (0–80 cm)	%
	C_{org_soil}	Organic carbon content (0–10 cm)	$g\ kg^{-1}$
	N_{tot_soil}	Total nitrogen content (0–10 cm)	$g\ kg^{-1}$
	C/N_{soil}	C:N ratio (0–10 cm)	–
	P_{inorg_soil}	Pool of inorganic phosphorus (0–10 cm)	$mg\ m^{-2}$
	P_{org_soil}	Pool of organic phosphorus (0–10 cm)	$mg\ m^{-2}$
	P_{tot_soil}	Pool of total phosphorus (0–10 cm)	$mg\ m^{-2}$
Atmospheric nitrogen deposition			
	DD_{NH3}	Dry deposition of ammonia	$kg\ N\ ha^{-1}\ year^{-1}$
	WD_{NH4}	Wet deposition of ammonium	$kg\ N\ ha^{-1}\ year^{-1}$
	WD_{NO3}	Wet deposition of nitrate	$kg\ N\ ha^{-1}\ year^{-1}$
	TD_{redN}	Total deposition of reduced nitrogen	$kg\ N\ ha^{-1}\ year^{-1}$
	TD_{oxN}	Total deposition of oxidized nitrogen	$kg\ N\ ha^{-1}\ year^{-1}$
	TD_N	Total deposition of nitrogen	$kg\ N\ ha^{-1}\ year^{-1}$
Landolt indicator values derived from vegetation surveys			
	$N_{Landolt}$	Mean Landolt ecological indicator value for nutrients (mostly nitrogen) (from 1: indicator of very infertile site to 5: indicator of rich site)	–
	$R_{Landolt}$	Mean Landolt ecological indicator value for soil acidity (from 1: indicator of very acidic soil to 5: indicator of basic soil)	–
	$F_{Landolt}$	Mean Landolt ecological indicator value for soil moisture (from 1: indicator of extreme dryness to 5: indicator of water-saturated soil)	–
	$L_{Landolt}$	Mean Landolt ecological indicator value for light (from 1: indicator of shade to 5: indicator of full light conditions)	–
	$T_{Landolt}$	Mean Landolt ecological indicator value for temperature (from 1: cold-indicator to 5: indicator of warm sites)	–

RESULTS

Description of Leaf Traits and Relationships Between Foliar Variables

At the plot level, the dry mass of 100 leaves ranged from 9.9 g (in ISO) to 15.7 g (in CHA I; **Table 3**; see **Table 1** for site abbreviations) and was, as expected, positively correlated with the average leaf area ($r = 0.64$, $p < 0.05$; see **Supplementary Table 2** for all correlations). Leaf area was lowest in ISO (10.8 cm² per leaf) and highest in NEU II (19.1 cm² per leaf), and correlated

positively with foliar Ca ($r = 0.74$, $p < 0.01$) and Mg ($r = 0.59$, $p < 0.05$) concentrations. SLA varied from 102 cm² g^{-1} (in SAI) to 141 cm² g^{-1} (in LAU), and increased with increasing N ($r = 0.65$, $p < 0.05$) and B ($r = 0.62$, $p < 0.05$) concentrations in foliage. It was negatively correlated with the foliar C/N ratio ($r = -0.78, p < 0.01$).

Foliar N concentrations (N_{fol}) ranged from 16.4 mg g^{-1} (in CHA II) to 22.9 mg g^{-1} (in VOR). The comparison of N_{fol} with the reference value range for adequate to optimal nutrition (18.7–23.2, Mellert and Göttlein, 2012) suggests that N is not

TABLE 3 | Leaf morphological and nutritional characteristics (average of five trees and standard deviation in brackets) at the 12 sites.

Site code	Morphology					Carbon and macronutrients							Lignin and phenols	
	SLA (cm² g⁻¹)	Leaf length (mm)	Leaf width (mm)	Dry mass (g/100 leaves)	Leaf area (cm²)	C (%)	N (%)	Ca (mg g⁻¹)	Mg (mg g⁻¹)	K (mg g⁻¹)	P (mg g⁻¹)	S (mg g⁻¹)	Lignin (%)	Phenol (mg g⁻¹)
BET	115	60.2	36.6	11.5	13.9	47.2	1.99	10.82	0.79	7.89	0.73	1.38	51.3	194
	(15)	(6.7)	(5.1)	(1.3)	(3.50)	(0.47)	(0.11)	(1.34)	(0.25)	(1.68)	(0.11)	(0.13)	(1.8)	(26)
CHA I	116	69.8	42.8	15.7	19.0	47.3	2.11	11.98	1.36	8.39	1.33	1.38	49.5	215
	(8)	(4.1)	(2.5)	(3.2)	(1.80)	(0.56)	(0.11)	(1.98)	(0.21)	(1.82)	(0.15)	(0.08)	(2.3)	(18)
CHA II	110	66.3	39.7	14.3	16.6	47.6	1.64	12.73	1.29	8.91	1.74	1.29	43.8	225
	(6)	(3.4)	(3.7)	(2.6)	(2.30)	(0.41)	(0.06)	(0.36)	(0.31)	(0.51)	(0.52)	(0.09)	(3.0)	(21)
ISO	106	53.7	32.2	9.9	10.8	49.1	2.14	4.36	1.03	7.41	1.08	1.46	49.6	204
	(9)	(9.9)	(6.2)	(3.6)	(3.70)	(0.42)	(0.27)	(1.41)	(0.33)	(2.14)	(0.13)	(0.17)	(1.8)	(14)
LAE	120	70.9	42.3	15.2	18.9	47.8	2.23	13.65	1.31	8.41	1.10	1.52	45.8	135
	(24)	(7.1)	(3.9)	(2.5)	(3.60)	(0.36)	(0.08)	(0.99)	(0.36)	(2.70)	(0.12)	(0.11)	(0.9)	(57)
LAU	141	65.7	39.0	11.0	15.8	47.2	2.16	9.73	1.54	6.39	1.34	1.41	43.6	189
	(24)	(3.1)	(1.8)	(1.7)	(1.40)	(0.17)	(0.19)	(1.92)	(0.25)	(1.72)	(0.14)	(0.12)	(3.2)	(39)
NEU I	120	69.3	41.2	14.4	17.9	47.4	2.13	15.17	1.55	9.00	0.98	1.35	46.1	165
	(10)	(7.6)	(4.8)	(2.4)	(4.00)	(0.62)	(0.21)	(3.61)	(0.44)	(1.71)	(0.09)	(0.10)	(3.4)	(59)
NEU II	122	69.4	43.8	14.4	19.1	47.3	2.05	16.98	1.69	7.69	0.87	1.44	49.1	130
	(16)	(9.4)	(5.1)	(4.1)	(4.40)	(0.46)	(0.14)	(3.25)	(0.19)	(0.81)	(0.06)	(0.07)	(7.4)	(46)
OTH	121	70.4	40.1	14.4	17.6	47.6	2.03	9.42	1.52	7.37	1.22	1.38	51.6	151
	(21)	(5.0)	(5.2)	(1.1)	(3.30)	(0.50)	(0.15)	(1.41)	(0.58)	(0.73)	(0.09)	(0.11)	(4.6)	(31)
SAI	102	60.8	37.9	14.8	14.7	47.1	1.91	11.22	1.42	8.42	0.85	1.46	42.5	135
	(16)	(4.6)	(2.5)	(3.2)	(1.90)	(0.58)	(0.10)	(1.72)	(0.61)	(2.28)	(0.09)	(0.09)	(1.9)	(63)
SCH	138	70.4	41.4	12.9	18.1	47.0	2.20	15.35	1.44	6.10	0.96	1.61	46.9	172
	(10)	(1.5)	(1.7)	(1.7)	(1.00)	(0.38)	(0.08)	(3.25)	(0.20)	(1.17)	(0.06)	(0.11)	(2.0)	(39)
VOR	125	65.3	37.8	11.7	15.2	47.8	2.29	3.38	0.86	6.07	0.97	1.56	47.6	210
	(20)	(5.9)	(2.4)	(2.1)	(1.90)	(0.48)	(0.17)	(0.87)	(0.20)	(1.64)	(0.09)	(0.08)	(6.4)	(37)
ANOVA p	0.012	0.008	0.028	0.010	0.008	0.001	0.001	<0.001	0.003	0.067	<0.001	0.001	0.002	0.001

	Microelements								Other					
	Al (µg g⁻¹)	B (µg g⁻¹)	Ba (µg g⁻¹)	Cu (µg g⁻¹)	Fe (µg g⁻¹)	Mn (µg g⁻¹)	Ni (µg g⁻¹)	Zn (µg g⁻¹)	δ¹³C (‰)	δ¹⁵N (‰)	C/N	Lignin/N	N/K	N/P
BET	50.1	14.6	4.2	6.8	58.6	67.9	<0.9	23.8	−29.60	−5.95	23.7	24.30	2.63	27.84
	(8.2)	(4.6)	(0.9)	(1.1)	(5.8)	(31.9)	(0.0)	(8.9)	(0.33)	(0.43)	(1.2)	(1.50)	(0.56)	(3.66)
CHA I	126.0	16.3	28.0	6.8	114.7	462.1	2.4	21.6	−29.22	−4.18	22.5	22.09	2.62	15.95
	(16.1)	(5.0)	(22.2)	(0.5)	(21.3)	(208.7)	(1.1)	(2.8)	(0.69)	(0.45)	(1.0)	(1.09)	(0.62)	(1.02)
CHA II	116.5	13.1	11.4	4.5	90.1	223.2	<0.9	26.1	−28.98	−8.43	29.0	24.93	1.84	10.12
	(8.7)	(4.9)	(2.4)	(0.5)	(5.0)	(89.2)	(0.9)	(9.0)	(0.95)	(0.66)	(0.9)	(1.83)	(0.06)	(2.49)
ISO	98.0	11.5	9.6	9.6	97.9	154.5	1.9	16.0	−29.36	−4.48	23.2	21.98	3.07	20.09
	(15.8)	(3.2)	(5.0)	(1.7)	(25.9)	(22.2)	(1.3)	(3.1)	(0.95)	(0.77)	(2.9)	(2.01)	(0.76)	(3.09)
LAE	68.9	27.2	8.6	7.1	73.5	121.7	<0.9	32.5	−28.64	−3.64	21.5	19.32	2.91	20.57
	(14.0)	(8.2)	(2.7)	(0.8)	(10.8)	(80.3)	(0.4)	(4.1)	(1.00)	(0.39)	(0.6)	(0.63)	(0.95)	(2.13)
LAU	94.3	21.6	30.0	7.4	84.8	968.9	3.9	31.1	−28.92	−6.16	22.0	19.02	3.51	16.21
	(21.7)	(2.1)	(6.0)	(1.0)	(13.4)	(322.5)	(1.1)	(2.7)	(0.58)	(0.29)	(2.0)	(2.32)	(0.67)	(1.44)
NEU I	78.7	18.7	7.5	7.3	75.1	103.9	<0.9	30.6	−27.83	−4.72	22.4	20.52	2.42	21.78
	(4.4)	(6.6)	(2.7)	(1.5)	(3.3)	(42.8)	(0.0)	(7.8)	(0.46)	(0.45)	(2.3)	(1.99)	(0.39)	(0.97)
NEU II	80.3	15.9	7.4	5.4	75.5	33.2	<0.9	27.5	−29.03	−4.92	23.2	22.40	2.67	23.58
	(10.4)	(3.9)	(2.4)	(0.9)	(7.0)	(5.9)	(0.0)	(1.7)	(0.69)	(0.54)	(1.6)	(3.12)	(0.13)	(1.30)
OTH	95.4	11.1	22.3	6.9	92.5	748.5	1.8	29.6	−29.25	−6.36	23.6	24.13	2.76	16.69
	(9.6)	(1.3)	(5.7)	(0.8)	(8.0)	(287.0)	(1.2)	(6.2)	(0.63)	(0.40)	(1.9)	(3.27)	(0.24)	(1.21)
SAI	132.9	9.2	18.8	6.0	102.7	84.4	<0.9	28.2	−28.12	−5.68	24.7	20.56	2.37	22.64
	(45.5)	(1.1)	(6.4)	(0.5)	(27.8)	(27.4)	(0.0)	(4.0)	(0.75)	(0.16)	(1.2)	(1.01)	(0.49)	(1.62)

(Continued)

TABLE 3 | Continued

	Microelements							Other					
	Al $(\mu g\,g^{-1})$	**B** $(\mu g\,g^{-1})$	**Ba** $(\mu g\,g^{-1})$	**Cu** $(\mu g\,g^{-1})$	**Fe** $(\mu g\,g^{-1})$	**Mn** $(\mu g\,g^{-1})$	**Ni** $(\mu g\,g^{-1})$	**Zn** $(\mu g\,g^{-1})$	**δ^{13}C** (‰)	**δ^{15}N** (‰)	**C/N**	**Lignin/N**	**N/K**	**N/P**
SCH	62.9	13.4	26.3	7.4	76.4	410.2	2.8	27.1	−29.39	−4.59	21.4	19.79	3.73	22.97
	(7.9)	(3.7)	(7.8)	(0.6)	(5.2)	(62.3)	(1.3)	(13.1)	(0.48)	(0.29)	(1.0)	(1.31)	(0.77)	(1.68)
VOR	102.4	15.3	13.8	7.7	105.4	1570.9	3.3	20.9	−30.53	−5.93	21.0	19.29	3.89	23.70
	(19.8)	(2.9)	(4.4)	(0.9)	(15.9)	(470.5)	(1.8)	(3.8)	(0.54)	(0.63)	(1.5)	(3.07)	(0.56)	(0.70)
ANOVA p	**0.002**	**0.001**	**<0.001**	**0.003**	**0.005**	**<0.001**	**<0.001**	**0.006**	**<0.001**	**0.002**	**<0.001**	**0.003**	**0.001**	**0.002**

ANOVA p gives the p-value of an analysis of variance among the sites.

limiting at our sites except in CHA II. Foliar N concentrations were positively correlated with foliar S ($r = 0.64$, $p < 0.05$), Cu ($r = 0.80$, $p < 0.01$) and Ni ($r = 0.66$, $p < 0.05$) concentrations.

Foliar P concentrations (P_{fol}) ranged from 0.73 mg g^{-1} (in BET) to 1.74 mg g^{-1} (in CHA II). P_{fol} was lower than the lower bound of the range for adequate to optimum nutrition (1.2 – 1.9, Mellert and Göttlein, 2012) at the majority of our sites, indicating that P is a limiting factor. The foliar N/P ratio was higher than the optimum range at all sites except LAU, OTH, CHA I and CHA II. At CHA II, P_{fol} was particularly high, N_{fol} low, and N/P_{fol} the lowest of all sites. Foliar Mg (Mg_{fol}) varied from 0.79 mg g^{-1} (in BET) to 1.69 mg g^{-1} (in NEU II), and was below the range for optimum nutrition (1.1 – 2.3, Mellert and Göttlein, 2012) at BET, VOR and ISO. VOR and ISO were also the sites with the lowest foliar Ca concentrations ($Ca_{fol} < 5$ mg g^{-1}, i.e., below the lower bound of the range for optimum nutrition of 6.7–14 mg g^{-1} according to Mellert and Göttlein, 2012). In contrast, Ca_{fol} was above the upper threshold at SCH, NEU I and NEU II. Foliar K concentrations (K_{fol}) ranged from 6.1 mg g^{-1} (in VOR and SCH) to 9.0 mg g^{-1} (in NEU I) and were mostly in the optimum nutrition range (6.1–9.7 mg g^{-1}, Mellert and Göttlein, 2012), as was the N/K ratio. Foliar Al and Fe concentrations were closely related to each other ($r = 0.89$, $p < 0.001$) and were particularly high at the three sites in Valais (SAI, CHA I and CHA II).

The phenol and lignin concentrations varied considerably, from 130–225 mg g^{-1} phenol and 42.5–51.6% lignin (**Table 3**). The lignin content was negatively correlated with δ^{13}C ($r = -0.68$, $p < 0.05$). Foliar δ^{13}C ranged from −30.5‰ (VOR) to −27.8‰ (NEU I) and correlated positively with K_{fol} ($r = 0.72$, $p < 0.01$) and the foliar Zn concentration Zn_{fol} ($r = 0.73$, $p < 0.01$). Foliar δ^{15}N ranged from −8.4‰ (CHA II) to −3.6‰ (LAE) and was weakly positively correlated with N_{fol} ($r = 0.50$, $p < 0.10$).

The PCA synthesized the correlations between variables fairly well and determined the leaf variables that differentiated the sites most clearly (**Figure 2**). The first two components of the PCA (Dim1 and Dim2) explained a major part of the leaf variables' variance in our data set (41.7%). Together with the following two components, 63.5% of the total variance was explained. Dim1 was related to Ca_{fol}, Mg_{fol} and leaf area, and it separated ISO and VOR, two sites on acidic soils with low Ca_{fol}, from the other sites. Dim2 was related to the N status of the leaves

(N_{fol}, δ^{15}N) and to S_{fol}, and mostly discriminated the CHA II site from the others.
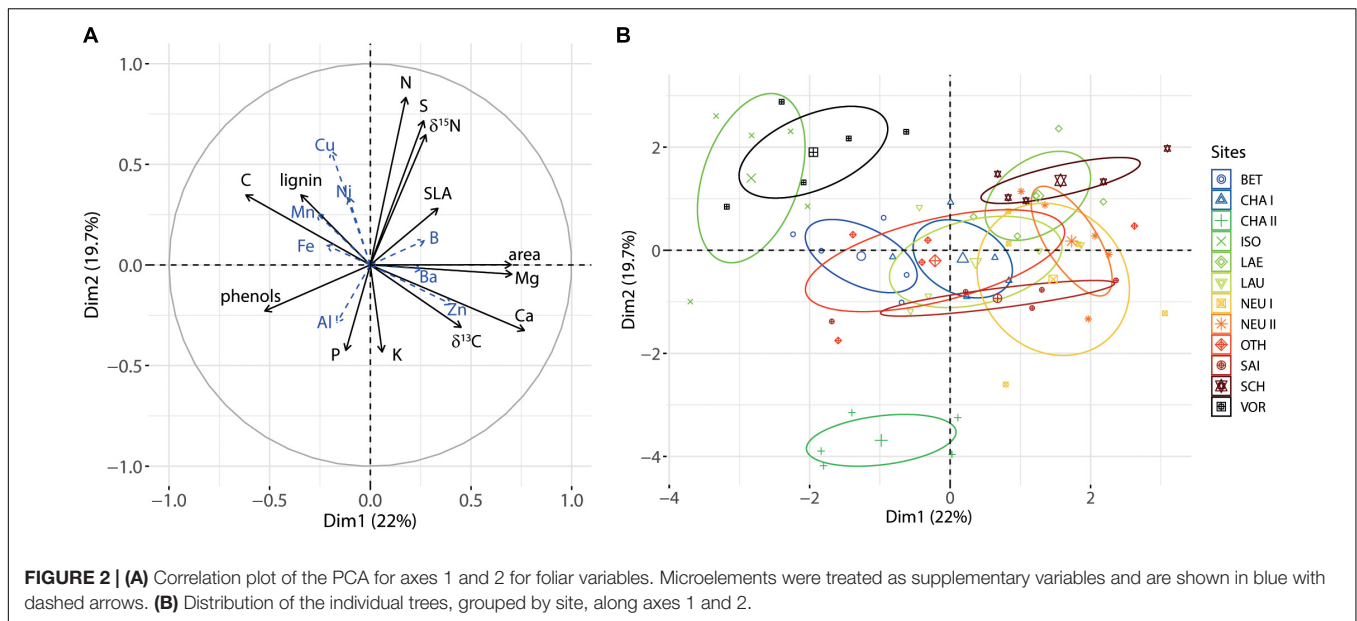
Relationships Between Leaf Traits and Environmental Variables
Leaf Morphological Traits
The dry mass of 100 leaves was negatively correlated with MAP ($r = -0.70$, $p < 0.05$; **Figure 3A**; see also **Supplementary Table 3** for all correlations) and AT/PT ($r = -0.59$, $p < 0.05$; **Figure 3B**). It tended to increase from low to high MAT ($r = 0.55$, $p < 0.10$; **Figure 3C**). The average leaf area was not correlated with MAP ($r = -0.38$, n.s.) or MAT ($r = 0.27$, n.s.) but tended to decrease with increasing AT/PT ($r = -0.52$, $p < 0.10$; **Figure 3D**) and increasing elevation ($r = -0.53$, $p < 0.10$; **Figure 3E**). Likewise, leaf width tended to negatively correlate with AT/PT ($r = -0.56$, $p < 0.10$) and leaf length tended to decrease with increasing elevation ($r = -0.59$, $p < 0.05$). SLA showed no significant correlation with MAP ($r = 0.34$, n.s.), AT/PT ($r = 0.34$, n.s.) or MAT ($r = 0.01$, n.s.). However, SLA decreased significantly with increasing elevation ($r = -0.70$, $p < 0.05$; **Figure 3F**).

Leaf Carbon and Nutrients
Foliar C concentrations showed no correlation with climatic variables (**Supplementary Table 3**). N_{fol} was positively correlated with MAP ($r = 0.59$, $p < 0.05$; **Figure 4A**) and also tended to increase with increasing AT/PT ($r = 0.52$, $p < 0.10$). It showed no significant relationship with established indicators of N availability for the vegetation, such as the C/N ratio of the topsoil ($r = -0.34$, n.s) or the vegetation-based indicator value $N_{Landolt}$ ($r = 0.23$, n.s). N_{fol} was, however, negatively correlated with soil pH ($r = -0.63$, $p < 0.05$; **Figure 4B**) and the indicator value $L_{Landolt}$ for light ($r = -0.69$, $p < 0.05$; **Figure 4C**), and positively correlated with the indicator value $F_{Landolt}$ for soil humidity ($r = 0.77$, $p < 0.01$; **Figure 4D**). CHA II, with its particularly low foliar N concentrations, was the driest (lowest $F_{Landolt}$ value across all sites) and most open site (highest $L_{Landolt}$ value) according to the species composition of ground vegetation. Finally, N_{fol} tended to increase with increasing N deposition ($r = 0.58$, $p < 0.05$ with WD_N, $r = 0.59$, $p < 0.05$ with TD_N). The closest relationship between foliar N and N deposition was found when the total deposition of the oxidized form of N (TD_{oxN}) was considered ($r = 0.68$, $p < 0.05$).

FIGURE 2 | (A) Correlation plot of the PCA for axes 1 and 2 for foliar variables. Microelements were treated as supplementary variables and are shown in blue with dashed arrows. **(B)** Distribution of the individual trees, grouped by site, along axes 1 and 2.

P_{fol} was not correlated with any of the (pedo-)climatic variables (MAP, MAT or AT/PT). There were few significant correlations between P_{fol} and the other environmental variables ($r = -0.52$, $p < 0.10$ with N_{soil}, $r = 0.68$, $p < 0.05$ with P_{inorg_soil}; **Figure 5A**). The foliar N/P ratio was also negatively correlated with P_{inorg_soil} ($r = -0.82$, $p < 0.01$ **Figure 5B**). None of the tested relationships between Mg_{fol} and environmental variables was significant. In particular, there was no correlation between Mg_{fol} and Mg_{soil}. There was a weak negative relationship between Ca_{fol} and AT/PT ($r = -0.51$, $p < 0.10$; **Figure 6A**). Foliar K concentrations (K_{fol}) were higher at drier than at wetter sites ($r = -0.69$, $p < 0.05$ with AT/PT, **Figure 6C**; $r = -0.66$, $p < 0.05$ with MAP). Ca_{fol} and K_{fol} increased significantly with increasing soil pH (**Figures 6B,D**), base cation concentrations and base saturation, and were negatively correlated with Al_{soil} and Fe_{soil}.

The foliar N/K ratio (N/K_{fol}) increased with increasing MAP ($r = 0.78$, $p < 0.01$) and AT/PT ($r = 0.73$, $p < 0.01$; **Figure 7A**), as a result of increasing N_{fol} and decreasing K_{fol} along these gradients. N/K_{fol} was strongly and negatively correlated with soil pH (**Figure 7B**), base cation content and base saturation. Correlations with the indicator value of ground vegetation revealed consistent patterns, with increasing N/K_{fol} with increasing indicator value $F_{Landolt}$ (**Figure 7C**) and decreasing N/K_{fol} with increasing $R_{Landolt}$. Consistent with the trends of increasing N_{fol} and decreasing K_{fol} along the N deposition gradient, N/K_{fol} increased with increasing N deposition ($r = 0.75$, $p < 0.01$ with WD_N, $r = 0.73$, $p < 0.01$ with TD_N; **Figure 7D**).

Among microelements, foliar Cu concentrations (Cu_{fol}) were positively correlated with AT/PT ($r = 0.72$, $p < 0.01$) and MAP ($r = 0.83$, $p < 0.001$). Cu_{fol} was also negatively correlated with soil pH, soil Ca concentrations and base saturation, and positively with soil Al. Cu_{fol} was positively correlated with WD_N ($r = 0.72$, $p < 0.01$).

Additional Leaf Traits

The phenol and lignin concentrations were not correlated with any of the environmental variables considered here (**Supplementary Table 3**). The $\delta^{15}N$ signature showed no significant correlation with climate, soil properties, indicator values of vegetation or N deposition. Along the precipitation gradient, the highest $\delta^{13}C$ values were found on dry sites ($r = -0.63$, $p < 0.05$ with AT/PT, $r = -0.57$, $p < 0.10$ with MAP). $\delta^{13}C$ was also positively correlated with the temperature indicated by the ground vegetation $T_{Landolt}$ ($r = 0.58$, $p < 0.05$), and negatively with WD_N ($r = -0.64$, $p < 0.05$).
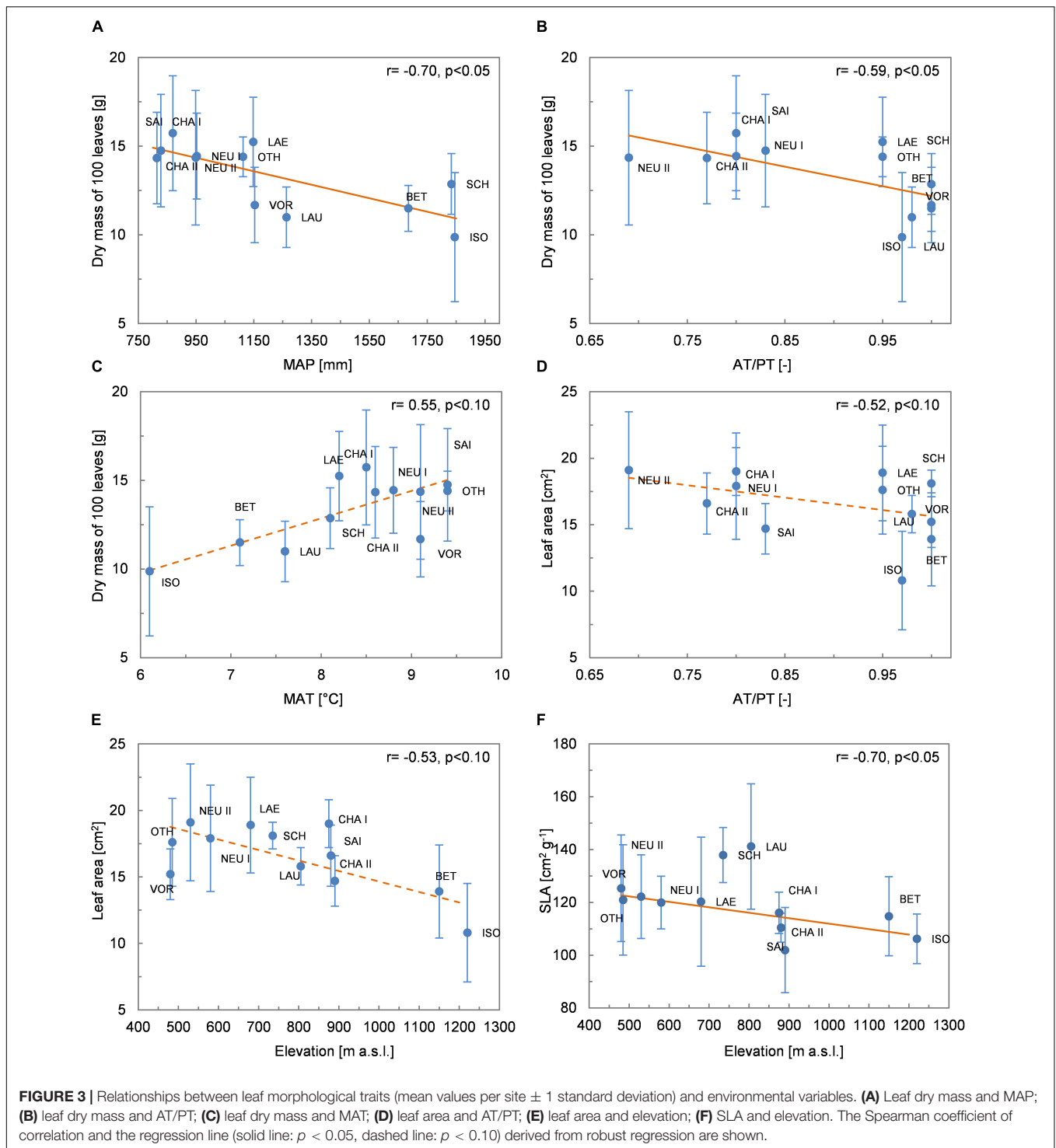
Multivariate Analyses

Plotting the environmental variables as supplementary variables on the first two PCA axes (**Figure 8**) synthesized the correlations between foliar and environmental variables and illustrated the superposition of the soil acidity and water availability gradient: soil base saturation (BS) or soil Ca content and MAT are plotted close to each other and in the opposite direction compared with MAP and AT/PT.

Exploratory linear regression models that included two or three predictors were found for two leaf traits only. The dry mass of 100 leaves was best explained by the base saturation of the soil (positive effect) and MAP (negative effect), while leaf area was best determined by the C/N ratio of the topsoil (negative effect) and MAT (positive effect) (**Table 4**).
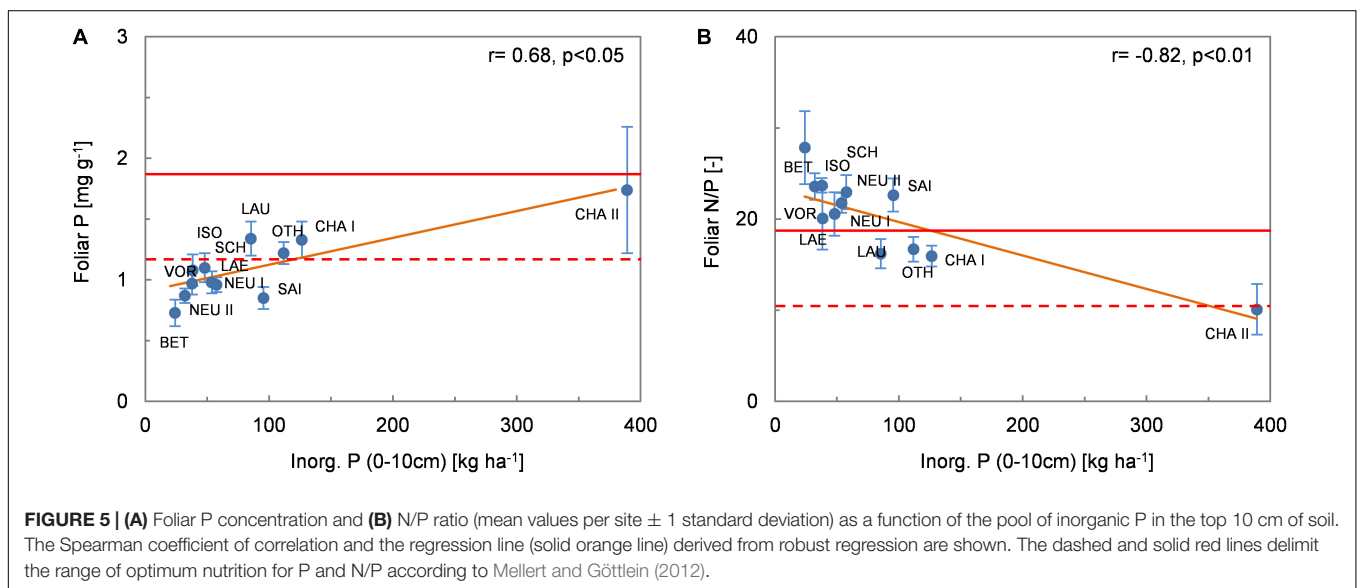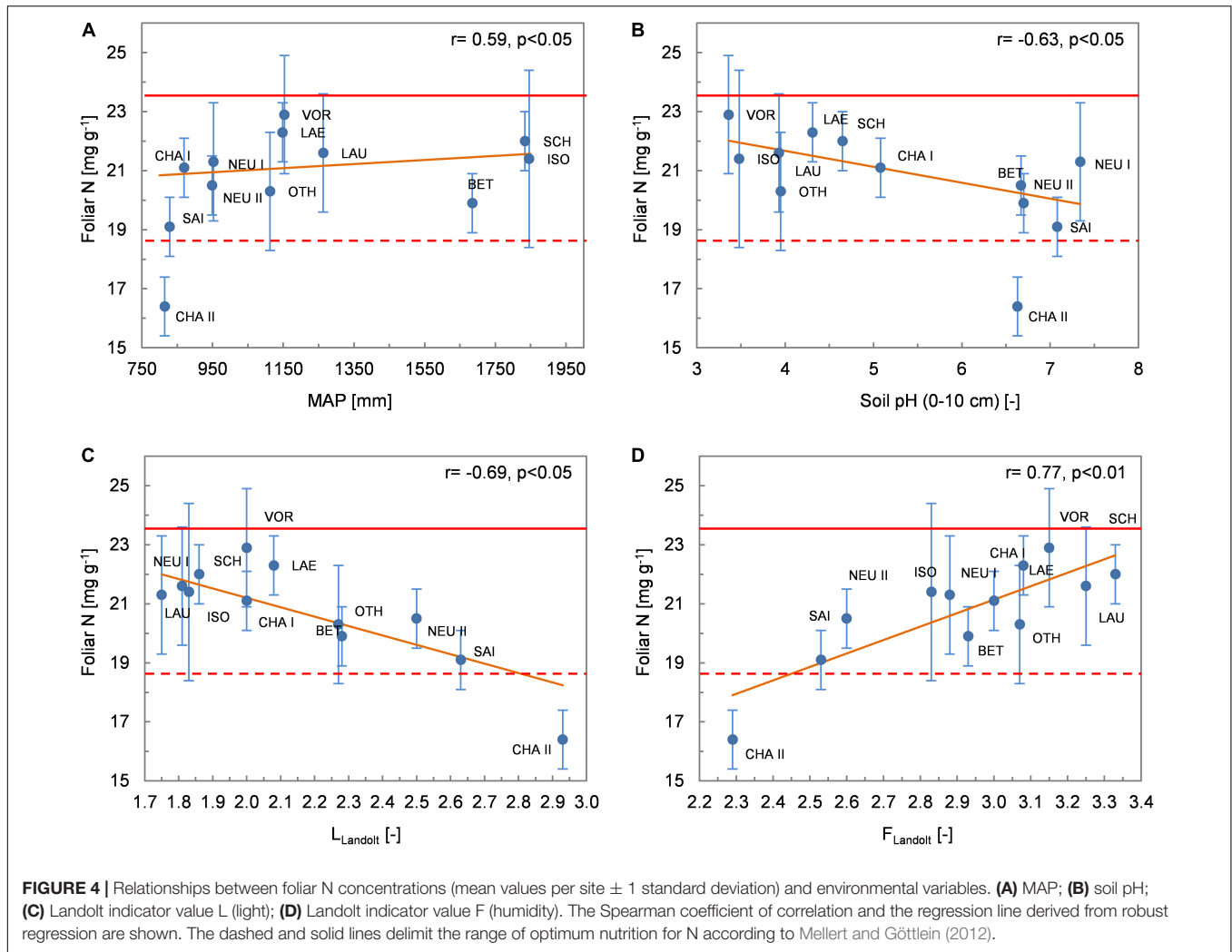
DISCUSSION

In view of the predicted increase in frequency and intensity of summer drought for Switzerland, the responses of foliage properties (nutrient concentrations, leaf size and specific leaf area) of beech trees to changes in water availability and soil properties along an MAP (or AT/PT) gradient were investigated
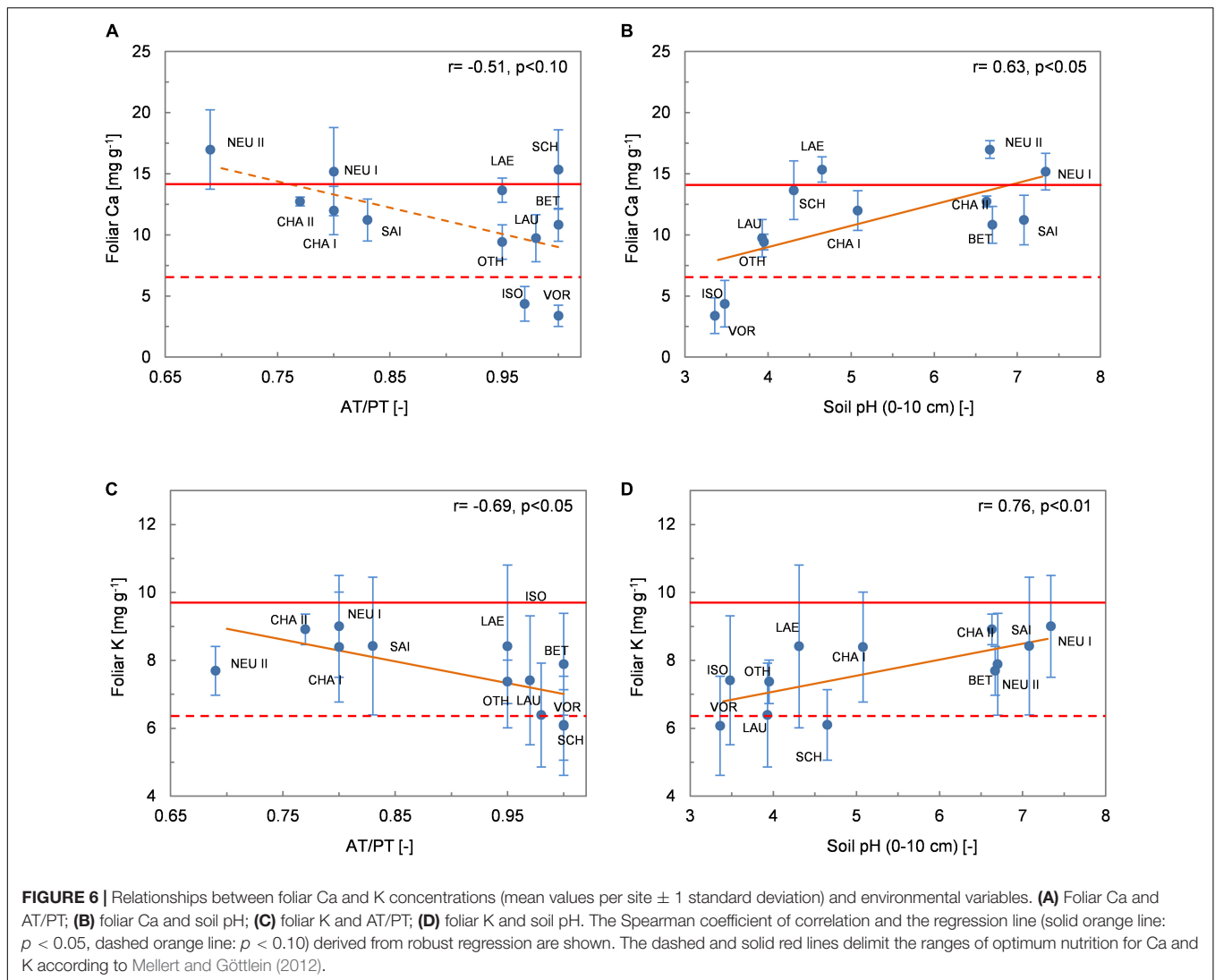
FIGURE 3 | Relationships between leaf morphological traits (mean values per site ± 1 standard deviation) and environmental variables. **(A)** Leaf dry mass and MAP; **(B)** leaf dry mass and AT/PT; **(C)** leaf dry mass and MAT; **(D)** leaf area and AT/PT; **(E)** leaf area and elevation; **(F)** SLA and elevation. The Spearman coefficient of correlation and the regression line (solid line: $p < 0.05$, dashed line: $p < 0.10$) derived from robust regression are shown.

based on 12 forest stands distributed throughout the country. It has already been reported that beech is comparatively vulnerable to summer drought and to rising temperatures (Geßler et al., 2007; Packham et al., 2012; Hanewinkel et al., 2013; Zimmermann et al., 2015). Higher temperatures and reduced precipitation during recent years have been identified as main contributors of

a recent growth decline in beech forest stands (Knutzen et al., 2017). The vulnerability of beech trees to such climatic changes might be associated with their large crowns and high water consumption (Leuschner and Ellenberg, 2017).

The results of the present study suggest that water availability, as assessed using long-term proxy variables, has effects on leaf
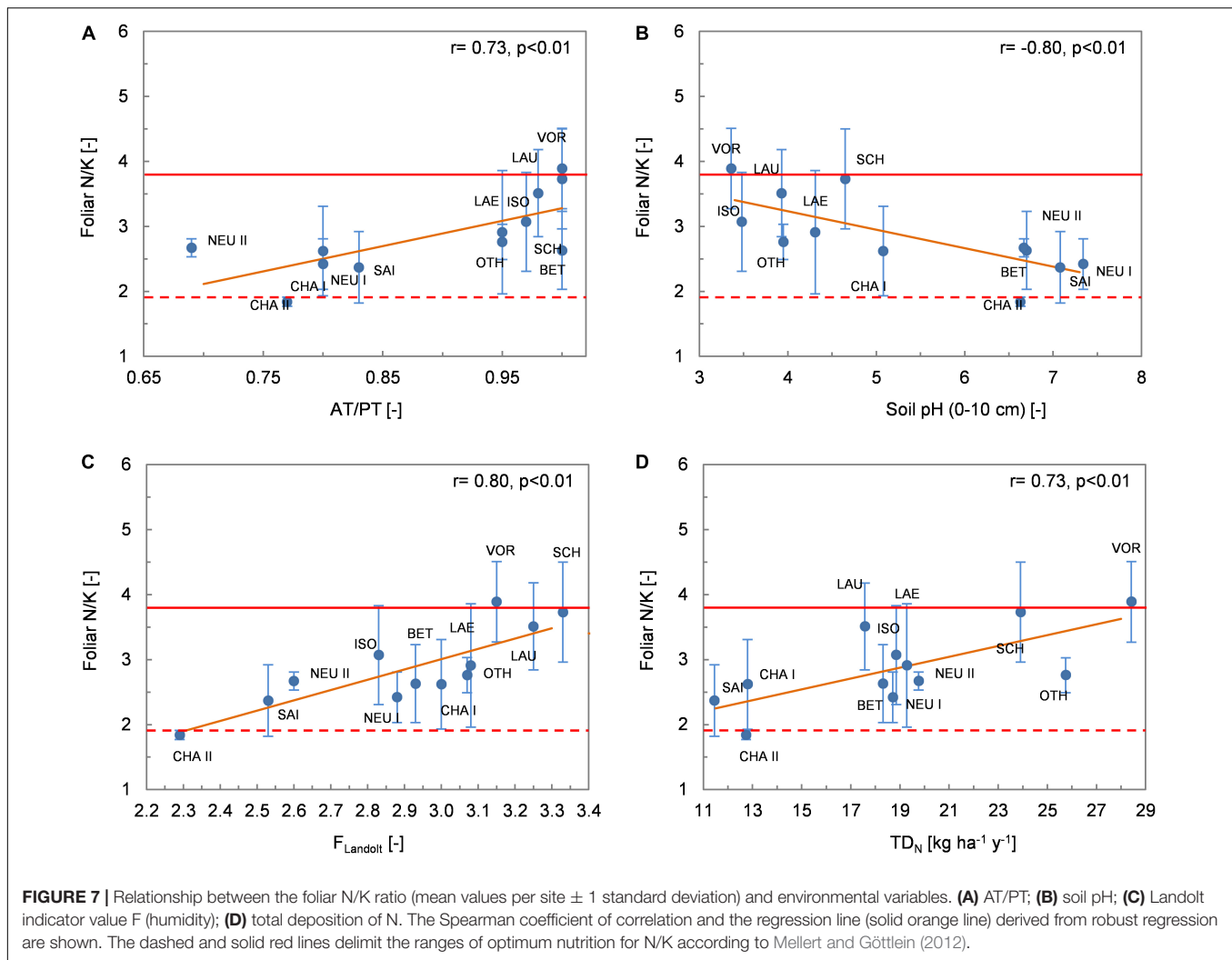
FIGURE 4 | Relationships between foliar N concentrations (mean values per site ± 1 standard deviation) and environmental variables. (A) MAP; (B) soil pH; (C) Landolt indicator value L (light); (D) Landolt indicator value F (humidity). The Spearman coefficient of correlation and the regression line derived from robust regression are shown. The dashed and solid lines delimit the range of optimum nutrition for N according to Mellert and Göttlein (2012).

FIGURE 5 | (A) Foliar P concentration and (B) N/P ratio (mean values per site ± 1 standard deviation) as a function of the pool of inorganic P in the top 10 cm of soil. The Spearman coefficient of correlation and the regression line (solid orange line) derived from robust regression are shown. The dashed and solid red lines delimit the range of optimum nutrition for P and N/P according to Mellert and Göttlein (2012).

FIGURE 6 | Relationships between foliar Ca and K concentrations (mean values per site ± 1 standard deviation) and environmental variables. **(A)** Foliar Ca and AT/PT; **(B)** foliar Ca and soil pH; **(C)** foliar K and AT/PT; **(D)** foliar K and soil pH. The Spearman coefficient of correlation and the regression line (solid orange line: $p < 0.05$, dashed orange line: $p < 0.10$) derived from robust regression are shown. The dashed and solid red lines delimit the ranges of optimum nutrition for Ca and K according to Mellert and Göttlein (2012).

morphology. We observed a significant reduction in leaf dry mass with higher precipitation (MAP) and AT/PT (**Figure 3**). A similar pattern, yet only marginally significant ($p < 0.10$), was observed for leaf area. These results seem contradictory with the trend of reduced leaf size with decreasing MAP reported in meta-studies (e.g., McDonald et al., 2003; Peppe et al., 2011; Moles et al., 2014) or with responses to water shortage observed in experiments using potted tree saplings (e.g., Knutzen et al., 2015). Our findings, on the other hand, show some consistency with the results reported by Meier and Leuschner (2008) in a precipitation gradient study comparable to ours, with 14 mature beech forest stands. These authors found that the average leaf size (area) was greater in dry stands than in moist stands. They argued that higher air temperature in May at the drier sites, in a period when soil water reserves are sufficient, stimulated leaf expansion, i.e., the phase of development that contributes most to the leaf surface area (Van Volkenburgh, 1999). The full size of the leaves is thus reached before the onset of drought conditions in summer. Favorable temperatures in spring could

likewise explain the relatively large leaf size of beech trees at our driest sites. Using the long-term average of mean air temperature in April and May did not improve the correlation between leaf area and temperature ($r = 0.28$, n.s., data not shown, compared with $r = 0.27$ for MAT). More importantly, however, continuous measurements of soil water potential at the driest sites of our sample set, which are also among the warmest sites, indicate non-limiting water availability in spring, in contrast to during the summer period (for an example, see the results for site SAI in **Figure 4** of Walthert and Schleppi, 2018). Another explanation could be that leaf growth has adapted to drought experienced in the past and that it can therefore be better maintained under conditions of low water availability (Knutzen et al., 2015).

We found no statistically significant relationships between SLA and climatic factors, although decreasing SLA with decreasing precipitation or water availability (Bussotti et al., 2000; Niinemets, 2001; McDonald et al., 2003; Poorter et al., 2009) and relationships with mean annual temperature (Moles et al., 2014) or soil fertility (Cunningham et al., 1999) have been reported in

FIGURE 7 | Relationship between the foliar N/K ratio (mean values per site ± 1 standard deviation) and environmental variables. **(A)** AT/PT; **(B)** soil pH; **(C)** Landolt indicator value F (humidity); **(D)** total deposition of N. The Spearman coefficient of correlation and the regression line (solid orange line) derived from robust regression are shown. The dashed and solid red lines delimit the ranges of optimum nutrition for N/K according to Mellert and Göttlein (2012).

several studies. The interaction among environmental factors and the covariation of gradients in our data set (e.g., soil acidity and water availability) make it difficult to detect systematic patterns. It is also much more difficult to identify clear relationships when a single species is considered rather than a large number of species over larger gradients, as is the case in the studies cited above. However, the relatively low SLA values at the dry and warm sites of CHA I, CHA II and SAI, in the Valais, are consistent with the hypothesis that species growing in dry environments often produce thicker leaves to minimize water loss (Wright et al., 2004). In addition, the low SLA value of the beech trees at the ISO site, with the highest MAP but also the lowest MAT across our gradient, could be explained by an increased thickness or density of the leaves as an adaptation to cold conditions (Körner et al., 2016). The high foliar C concentrations at ISO (**Table 3**) might be related to a particular chemical composition of the leaves, such as an increase in structural C compounds as a strategy to overcome cold-related stress, but this was not analyzed further within the present study. SLA and leaf length showed negative correlations with elevation in our data set (**Figure 3**),

in line with findings from the literature (e.g., Hovenden and Vander Schoor, 2004). Ecological changes along elevational gradients are mostly interpreted as being driven by variations in temperature, which generally decreases linearly with increasing elevation. However, the fact that SLA and leaf length correlate more closely with elevation than with MAT across our beech stands suggests that other environmental factors, co-varying with elevation, might also play a role (e.g., irradiance, Hovenden and Vander Schoor, 2006). Furthermore, in our study, elevation is very closely and negatively correlated with dry deposition of N, in particular in its reduced form (NH_3) ($r = -0.94$, $p < 0.001$; **Supplementary Table 1**).

Specific leaf area was positively correlated with foliar N concentrations in our data set, and this relationship has been widely observed in the literature, together with a positive relationship of SLA and N_{fol} with the photosynthetic capacity of the leaves (e.g., Reich et al., 1999). We found that foliar N concentrations increased from dry to moist sites, along a gradient of increasing MAP and increasing Landolt indicator value F for soil humidity (**Figure 4**). Lower nutrient concentrations in foliage
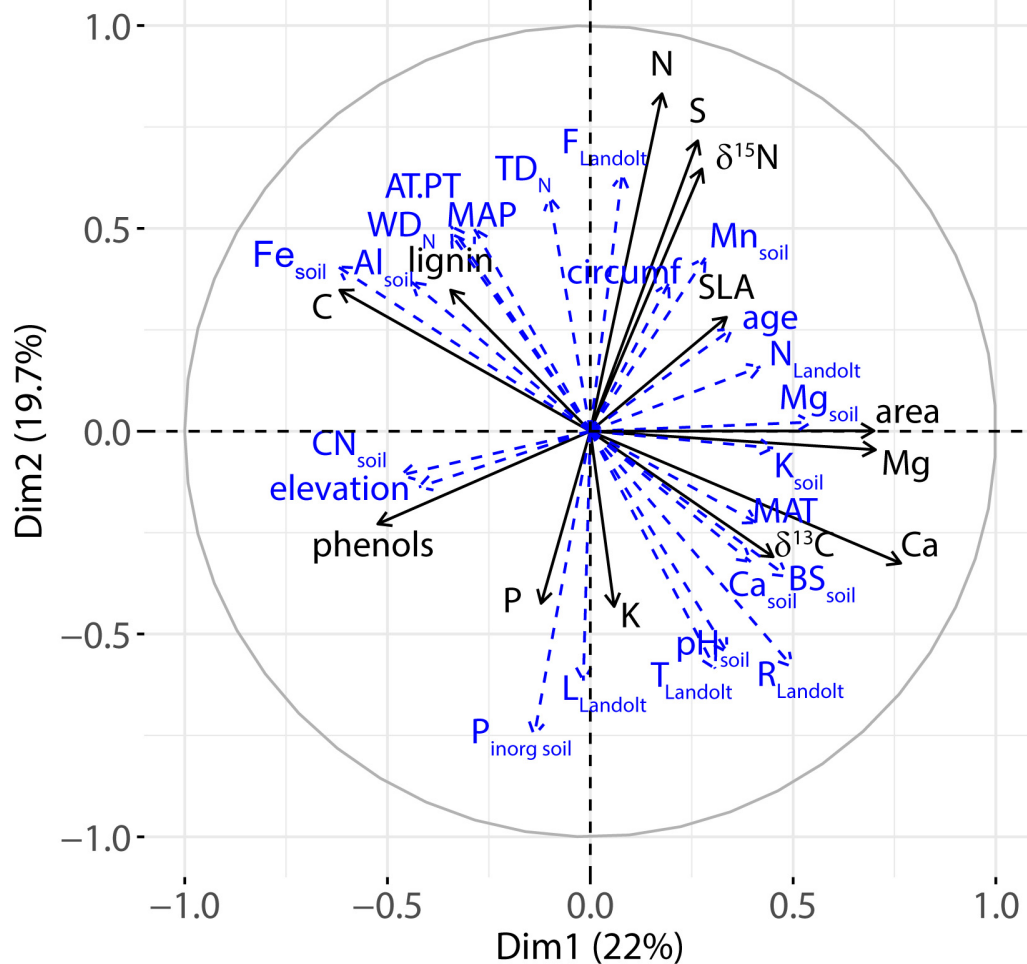
FIGURE 8 | Variable correlation plots of the PCA for axes 1 and 2. Environmental variables (soil, climate, N deposition, Landolt indicator values) were treated as supplementary variables and are shown in blue with dashed arrows.

at sites with reduced water availability could be explained by a lower supply of nutrients to the roots due to reduced mass flow and diffusion in the pedosphere, or to reduced microbial activity and a lower decomposition rate (see review by Kreuzwieser and Gessler, 2010). However, we observed the inverse pattern for foliar K, which decreased with increasing MAP and AT/PT (**Figure 6**), and we found no significant correlation between foliar P or Mg and climatic factors. N_{fol} is clearly influenced by multiple factors, among which atmospheric deposition of N seems to play a role, as indicated by the correlation analysis and the results of the PCA. A considerable fraction of N in trees is taken up through the roots, but NH_4^+ and NO_3^- dissolved in precipitation (wet deposition) and gaseous forms of N, in particular NH_3 (dry deposition), can be directly taken up by the forest canopy (e.g., Harrison et al., 2000) and contribute significantly to the annual N requirement for growth (e.g., Sievering et al., 2007). Some other studies have shown a positive relationship between N deposition and foliar N concentrations of trees (Waldner et al., 2015), although this relationship is

usually less clear for beech than for coniferous species such as Norway spruce (e.g., Braun et al., 2018). We further speculate that the negative correlation observed between N_{fol} and $L_{Landolt}$, the indicator for light conditions below the forest canopy, might be explained by better growth and denser foliage at sites well supplied with N. The positive effect of a lower soil C/N ratio on leaf area observed in the linear regression analysis (**Table 4**) tends to support this hypothesis. On the other hand, we observed no significant relationship between the LAI of the forest stand and N_{fol} (**Supplementary Table 3**), but this could be due to the use of modeled rather than measured LAI values in our analysis.

While N_{fol} tended to be positively influenced by N deposition, K_{fol} decreased and N/K_{fol} increased along the N deposition gradient (**Figure 7**). This pattern might be ascribed to the negative effect of increased N on the mycorrhizal communities reported in several studies (e.g., de Witte et al., 2017; van der Linde et al., 2018) and the resulting impaired nutrient uptake. Braun et al. (2018), for example, observed a significant decrease in K concentration in beech foliage with decreasing

TABLE 4 | Results of significant linear regression models run on foliar traits (for abbreviations of variables see **Table 2**).

(a) Dry mass of 100 leaves (g)

Mean	R^2	Root mean square error
13.3	0.77	1.01

Source	Degrees of freedom	Sum of squares	Mean square	F	p(f > F)	Parameter estimate	Standard error
BS	1	8.4	8.4	8.15	0.019	0.023	0.008
MAP	1	12.1	12.1	11.83	0.007	−0.003	0.001
Model	2	30.4	15.2	14.80	0.001		
Error	9	9.2	1.0				
Corrected total	11	39.6					

(b) Leaf area (cm^2/leaf)

Mean	R^2	Root mean square error
16.5	0.79	1.26

Source	Degrees of freedom	Sum of squares	Mean square	F	p(f > F)	Parameter estimate	Standard error
C/N	1	27.3	27.3	17.14	0.003	−0.510	0.123
MAT	1	27.9	27.9	17.50	0.002	1.602	0.383
Model	2	54.8	27.4	17.19	0.001		
Error	9	14.3	1.6				
Corrected total	11	69.2					

diversity of ectomycorrhizal root tips. However, as N deposition is very closely related to climatic variables (e.g., $r = 0.94$, $p < 0.001$ between WD_N and MAP), it is difficult to single out the effect of N deposition on nutrient foliar concentrations in our data set. Nevertheless, knowing that N and S deposition are often correlated, the rather close relationship between N_{fol} and S_{fol} might be supportive of an influence of deposition on foliar concentrations.

Although K_{fol} changes across various gradients, K nutrition is, according to the optimum range of foliar concentrations available from the literature, satisfactory at practically all of our sites. Satisfactory K nutrition helps to alleviate drought stress at dry sites through the role of K^+ in stomatal regulation and the importance of this cation for the osmotic potential in the vacuoles (Marschner, 1995). This could explain why beech can maintain its presence at the driest sites of our gradient. On the other hand, except where pools of inorganic P in the top 10 cm of soil were large, we measured foliar P concentrations indicative of P limitation at most of our sites. This observation is in line with other studies in Switzerland (Braun et al., 2018) and elsewhere in Europe (Jonard et al., 2015; Talkner et al., 2015). P_{fol} was correlated with very few variables and, in particular, did not show a significant correlation with N deposition in our data set. A widespread hypothesis is, however, that N in excess due to atmospheric deposition contributes to impaired P uptake by trees through changes in the mycorrhizal community (Talkner et al., 2015).

Regarding microelements, one of the strongest correlations between climatic factors and foliar concentrations was observed for Cu (positive correlation between Cu_{fol} and MAP or AT/PT,

Supplementary Table 3). We suspect that this correlation was a consequence of the superposition of the soil acidity and water availability gradients (negative correlation between soil pH and MAP or AT/PT), as copper availability is known to be substantially affected by soil pH, decreasing 99% for each unit increase in pH (Mortvedt, 2000).

Lastly, no clear pattern was found between foliar $\delta^{15}N$ and climatic factors. In particular, the positive correlation observed by Meier and Leuschner (2014) between $\delta^{15}N$ and MAP was not apparent in our dataset. Furthermore, foliar $\delta^{15}N$ is expected to increase with increasing N deposition and decreasing C/N ratio of the forest floor, and it is expected to be a comprehensive indicator of the N status of the ecosystem (Pardo et al., 2006). However, in our data set, the only variable that was significantly correlated with foliar $\delta^{15}N$ was N_{fol}. This correlation has been observed in several earlier studies (Craine et al., 2009). Regarding $\delta^{13}C$, the negative relationship with AT/PT and MAP that we observed is in line with findings from the literature. Drought stress caused by low precipitation or high evaporation results in stomatal closure, which results in a less efficient discrimination of ^{13}C and finally in a less negative $\delta^{13}C$ value (Garten and Taylor, 1992; Wang et al., 2013).

CONCLUSION

In our study, the average leaf dry mass and leaf area tended to decrease with increasing water availability (using MAP or AT/PT as indicators), in contrast to what we expected. We found no linear relationship between SLA and long-term

(pedo-)climatic factors. At our driest sites, however, SLA tended to be lower, which partially supports our initial hypothesis. Regarding macronutrients, a positive correlation was found between foliar N and MAP, while the opposite was true for K. Our second hypothesis (less favorable foliar nutrient status at sites with lower water availability) was therefore not supported for all nutrients. Owing to the small number of sites relative to the number of variables studied, correlation analyses have a limited statistical power and must be interpreted with caution. Furthermore, collinearities between environmental factors in our data set make it difficult to single out the effect of water availability on leaf traits, as e.g., the gradient in soil acidity is more or less overlaid on our MAP and AT/PT gradient. We therefore suggest testing our findings using additional sites, such as those of the ICP Forests Level II network in other European countries. Still, a major advantage of our data set is the broad gradient of water availability covered: at Schänis and Isone, water availability is generally high, also in the context of Europe as a whole, while at two of our sites (Saillon and Chamoson II) at the dry end of the gradient, water shortage happened to be so severe that some of the beech trees died in late summer 2017.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

IB, AT, and MSa conceived and designed the study. LW, SZ, KL, PW, and MSc provided data and input for the data evaluations. AT, MSa, PS, and MA analyzed the data. All authors contributed to the writing of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2020.00019/full#supplementary-material

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