Plant-soil interactions determine ecosystem aboveground and belowground processes in primary dune ecosystems

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Chapter 4

Plant community responses to alterations in soil abiotic and biotic conditions are decoupled for above- and belowground traits

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Accepted by Journal of Ecology

Abstract

- Plant functional traits are increasingly recognized as being impacted by soil abiotic and biotic factors. Yet, the question to what extent community-level aboveground and belowground trait relationships are affected by soil conditions remains open.
- In a field experiment in dune grassland, we quantified the responses of both community-level leaf and root traits to changes in soil abiotic and biotic conditions using soil inoculation by living and sterile soil inocula originating from different ecosystems.
- Altered soil conditions resulted in a strong decoupling in responses of community-level leaf and root traits. Changes in soil abiotic conditions imposed by soil inoculation were more important in determining the decoupling of the leaf vs root relationships than additions of soil biota. Altered soil abiotic factors influenced both leaf and root traits at the community level and caused the entire community-level trait spectrum to shift, while experimental additions of living soil inocula only significantly influenced root traits towards longer and thinner roots.
- Our results bring direct evidence that, at a plant community level, the dynamics of plant aboveground traits are not informative of belowground traits. Particularly, belowground abiotic processes are a major driver of commonly observed trait spectra.

4.1 Introduction

Plant traits reflect the evolutionary and community assembly processes as influenced by abiotic and biotic factors (Westoby & Wright 2006; Valladares *et al.* 2007). Plants adapt to different abiotic conditions by adjusting multiple aspects of carbon and nutrient allocation, architecture, morphology and physiology (Van Kleunen & Fisher, 2001; Nicotra *et al.*, 2010). A growing body of studies indicates that plant functional traits values strongly depend on soil abiotic properties such as soil moisture, texture and nutrient availability (Gross *et al.* 2008; Ordoñez *et al.* 2010; Maire *et al.* 2015; Bergmann *et al.* 2016; Freschet *et al.* 2017). It has also been reported that many phenotypic properties of plants are derived from the interplay between the plant and its soil microbial associates (Friesen *et al.* 2011; Weigelt *et al.* 2021). Soil microbes interact with plants, intimately affecting the plant's capacity to acquire nutrients, uptake water and tolerate stresses (van der Heijden *et al.*, 2008; Bardgett & van der Putten, 2014). The interactions between plant and soil communities thus may affect plant functional trait expression by modifying plant responses to environmental stresses, and resource acquisition (Lau & Lennon 2012; Baxendale *et al.* 2014; Kulmatiski *et al.* 2017; Petipas *et al.* 2021; Xi *et al.* 2021).

The impacts of soil biotic and soil abiotic factors commonly interact in regulating plant growth (Bennett & Klironomos 2019; Kostenko & Bezemer 2020). Negative plant-soil interactions could play a central role in early communities which are generally characterized by resource-acquisitive traits on nutrient-poor soils during primary succession (Castle *et al.* 2016), while positive interactions have been shown to affect plant communities at later successional stages generally characterized by resource-conservative plant traits in competitive environments (De Deyn *et al.* 2004a; Kardol *et al.* 2006; Carbajo *et al.* 2011; Cortois *et al.* 2016). Therefore, it is expected that the variations in soil nutrient availability may interact with soil communities in modulating plant growth and plant defense through shaping plant trait values (Porazinska *et al.* 2003; Bjørnlund *et al.* 2012). For instance, the effects of beneficial soil microbes including plant growth-promoting bacteria (PGPB), arbuscular mycorrhizal fungi (AMF) and rhizobia on the host plant traits depend on the nutrient availability and forms in agricultural crop species (Wang *et al.* 2011; Saia *et al.* 2020). Yet, even though soil properties are recognized to be critical mediators of plant functional traits, the nature of the interactions between soil abiotic factors and soil biota on plant functional traits remains poorly studied due to the difficulty to experimentally modify belowground communities.

Based on the whole-plant economic traits (Poorter *et al.* 2014; Reich 2014), it is generally assumed that there are correlations between leaf and root traits to maximize the efficiency of obtaining and utilizing limited resources (Kramer-Walter *et al.* 2016). Indeed there is a growing body of evidence that at the level of individual plant species significant correlations exist between aboveground and belowground traits, such as specific leaf area (SLA) and

specific root length (SRL) (Liu *et al.* 2010; Fort *et al.* 2013), and tissue density of leaves and roots (Craine *et al.* 2001; Craine & Lee 2003; Fort *et al.* 2013). Nevertheless, recent studies demonstrate that root resource strategies are not fully consistent with leaf resource strategies across grasses, forbs and woody plant species (Kembel & Cahill 2011; Comas *et al.* 2014; Bergmann *et al.* 2016). Root trait complexity might result from the range of belowground resource uptake strategies that can be employed (Kramer-Walter 2016; Bergmann *et al.* 2017; Ma *et al.* 2018). In contrast to aboveground leaf carbon acquisition by photosynthesis being conducted exclusively by plants, the belowground parts of many plant species can outsource resource acquisition to associated soil microbes (Bardgett *et al.* 2014; Bergmann *et al.* 2020). For example, the symbiosis between arbuscular mycorrhizal (AM) fungi and plants can shape root traits including root cortical area and diameter, to provide intra-radical habitats for AM fungi (Brundrett 2002; Weemstra *et al.* 2016; Bergmann *et al.* 2020). This suggests a potential role of soil biota in driving certain aspects of root trait variation that are not mirrored in leaf traits, causing a decoupling of relationships between aboveground and belowground traits (Weemstra *et al.* 2016; Isaac *et al.* 2017; Laliberté 2017; Wang *et al.* 2017). Furthermore, the dynamics of functional traits of individual plant species is not necessary predictive for the dynamics of plant community-level traits. The latter, f.i. community-weighted mean trait values accounts for the variation in traits as well as for the between-species interactions and community composition. Yet, the extent to which entire soil communities modify these aboveground and belowground trait correlations is understood poorly.

This study aims to elucidate the responses of plant community traits to the alternation in both soil abiotic and biotic conditions, and especially how these responses influence the relationship between community-level leaf and root traits. To address this question, we manipulated the soil conditions by introducing different types of living and sterilized soil inocula originating from distinct dune ecosystems (primary dune, dune grassland and dune forest) in a newly established dune plant community. This setup allowed us to explicitly test the effect of changes in both abiotic and biotic soil conditions on the plant community (Middleton & Bever 2012; Wubs *et al.* 2016). We have sown a standardized mixture of seeds of plant species in the inoculated soils and after 3 years assessed plant community-level traits in each community to examine their responses to the different soil inoculation treatments.

4.2 Materials and Methods

- *Experimental design*

A detailed outline of experimental design is presented in general introduction of this thesis (Chapter 1). In short, the TERRA-Dunes experiment is a long-term filed experiment where the soil biotic and abiotic properties were manipulated through the addition of soil inocula. Soil inocula were collected from different donor dune ecosystems (Figure 1.3), including

primary dunes, dune grasslands and dune forests. Further, in order to examine the effects of soil biotic conditions on plants, half of the soil inocula was sterilized with gamma radiation (>25 KGray gamma radiation, Isotron, Ede, the Netherlands).

- *Plant functional trait measurements*

The absolute percentage cover of each plant species was recorded visually within each plot (2×2 m) in July 2020. For each species, aboveground and belowground plant traits were measured following standard trait measurement protocols (Cornelissen *et al.* 2003). Based on this vegetation survey, we selected those plant species that together comprised the top 80% of the total species cover in each plot. For selected species in each plot, we randomly sampled 15-20 matured, undamaged and unshaded leaves from at least 5 individuals in each plot. The following leaf traits were selected for the assessment: specific leaf area (SLA), leaf thickness (LT), leaf dry-matter content (LDMC), leaf carbon content (LCC), leaf nitrogen content (LNC) and leaf phosphorus content (LPC). These traits are most tightly related to the plant economic spectrum. Fresh leaves were weighed and scanned using a Cannon LiDE 210 scanner. Leaf area was obtained by analysis with software ImagineJ. Leaf thickness was measured at 2 points on the leaf, avoiding midribs, using a high precision caliper (+/- 0.01 mm). Thereafter, the leaf samples were oven-dried at 65°C for 48 h and weighted and then ground to fine powder. Specific leaf area was calculated as leaf area divided by its dry mass. Leaf dry-matter content was obtained as the ratio of leaf dry mass to fresh mass. Leaf C and N contents were analyzed using a Flash EA 1112 elemental analyzer (Thermo Scientific, Rodana, Italy). Leaf P content was determined using a UV/visible spectrophotometer after acid digestion with a 1:4 mixture of 37% (v/v) HCl and 65% (v/v) HNO_3 (Murphy & Riley 1962). For each selected plant species, we calculated the mean values of the plant leaf traits, and we calculated the relative abundance of each species in each plot as a ratio of plant cover of a given species to the community cover in plot. These relative abundances were used to calculate the community-weighted means (CWM) of the foliar traits.

The root systems of plants are intertwined in soil, and therefore we directly sampled the community-weighted mean traits. We took a composite measure of root traits from the mixed root systems collected from four soil cores (ø=3.5 cm, h=10 cm) in each plot. Then roots were carefully washed under tap water to allow the separation of roots by flotation using sieve stacks. Dead roots were separated by visual clues. 0.1 g fine roots were randomly selected and stored in 50% ethanol. AMF structures were stained with Trypan blue using a standard protocol (Robertson *et al.* 1999). Roots were cleared with 5% KOH solution in a 75 °C water bath for 30 minutes. Roots were then acidified in 1% HCl solution for 30 minutes and subsequently stored in 0.01% Trypan blue for 30 minutes in a 75 °C water bath. Roots were stored in 50% glycerol for microscopic investigation. The percentage of AM fungal root colonization (AMFC) was estimated according to the grid line interaction method (McGonigle *et al.* 1990). The remaining fresh roots were weighed and scanned on a Cannon

LiDE 210 scanner. Thereafter, the root samples were weighted after oven-drying at 65°C for 48 h and ground to fine powder. Root C and N contents (RCC, RNC) were analyzed using an elemental analyzer (Thermo Scientific, Rodana, Italy). Root P content (RPC) was determined using a UV/visible spectrophotometer after acid digestion. Total root length, volume and average diameter (AD) were determined using the scanned images with the software of WinRhizo (Regent Instruments, Quebec, Canada). Specific root length (SRL) was calculated as root total length divided by its dry mass. Root tissue density (RTD) was calculated as root volume divided by its dry mass. All trait abbreviations are listed in Table 4.1.

- *Soil sampling*

Soil samples were collected from all plots on September 10, 2020 and sieved (2 mm mesh size) in lab. Soil samples were separated into two parts for the measurement of soil abiotic properties and soil microbial composition following the protocols in supplementary materials. The complete set of results for the soil chemical results is presented in Table S3-2.

- *Data analysis*

To enable the application of a two-factor statistical analysis, all 24 control plots were a-priori randomly assigned to either living or sterile soil inocula. Pairwise trait relationships were assessed using Pearson's correlation test. We used principal component analysis (PCA) to visualize the axes of main variation in CWM trait values. Prior to PCA, missing data were replaced with the mean of each variable. Permutational multivariate analysis of variance (PERMANOVA) was used to test the effect of the soil inoculation treatments on the variation in CWM traits, based on a Bray-Curtis dissimilarity matrix in R using the package "vegan". In order to further evaluate the effects of soil inoculation treatments on individual CWM traits, a two-way ANOVA was run to test the effects of different types of soil inocula and soil sterilization treatments on CWM traits. Model assumption of normality and homoscedasticity were checked on the model residuals (Kozak & Piepho 2018) and variables were transformed when necessary to meet the assumption of model residuals. Effect size of the analyses was calculated using the function "eta_squared()". In case the effects of model parameters were significant in an ANOVA, a post-hoc test was performed using the lsmeans package, with the Turkey method for p-value adjustment (Lenth 2016).

4.3 Results

- *Relationship between root and leaf traits under different soil inoculation treatments*

Pairwise relationships between all CWM leaf traits and between most root traits were strong and highly significant (Table 4.2). All leaf traits were positively related to one another. All relations between root morphological traits were significant. SRL was negatively related to AD (r = -0.79; p<0.01) and RTD (r = -0.81; p<0.01). The root chemical trait associated with

resource acquisition, RNC, was significantly positively related to SRL (r = 0.43; p<0.01), RPC (r = 0.33; p<0.01) and AMFC (r = 0.32; p<0.01), and significantly negatively with AD (r = -0.32; p<0.01), RTD (r = -0.51; p<0.01) (Table 4.2). There were almost no significant relationships between leaf traits and roots traits apart from a low correlation between LNC and RNC (r = - 0.24; p=0.02), LPC and AD (r = 0.22; p=0.03), RTD (r = 0.22; p=0.04).

The main variation among all 13 CWM traits was visualized by a PCA. The first and second axes accounted for 26.9% and 22.4% of the total variation of plant community-level traits, respectively (Figure 4.1; Table S4-1). The first axis of this PCA primarily reflected differences in community-level root traits, whereas the second reflected differences mainly in community-level leaf traits except for RPC (Figure 4.1). Qualitatively, all the leaf traits covaried along with the leaf economics spectrum, while root traits covaried along an independent trait spectrum (Figure 4.1). Additional PCA analyses separately for plots with living or sterile soil inocula also showed orthogonal response patterns of leaf and root traits (Figure 4.2). These results strongly suggest that the leaf traits are orthogonal to root traits in response to alternations in soil conditions, and the orthogonality is driven by the other factors than presence or absence of soil biota.

Table 4.1 List of 13 measured CWM traits, as well as their descriptive statistics.

Trait	Abbreviation	Unit	Mean (s.d.)	Range
Leaf thickness	LT	mm	0.23 (0.04)	0.16-0.43
Specific leaf area	SLA	$cm^2\ g^{-1}$	124.91 (22.30)	70.86-203.25
Leaf dry matter content	LDMC	$mg\ g^{-1}$	173.02 (27.33)	114.88-256.12
Leaf carbon content	LCC	%	33.88 (3.86)	24.82-43.42
Leaf nitrogen content	LNC	%	1.59 (0.26)	1.00-2.16
Leaf phosphorus content	LPC	%	0.19 (0.04)	0.08-0.31
Average diameter	AD	mm	0.35 (0.04)	0.25-0.49
Specific root length	SRL	$m\ g^{-1}$	82.22 (37.64)	12.95-172.03
Root tissue density	RTD	$g\ cm^{-3}$	0.16 (0.08)	0.08-0.42
Root carbon content	RCC	%	50.33 (2.55)	44.27-59.03
Root nitrogen content	RNC	%	2.14 (0.31)	1.18-3.11
Root phosphorus content	RPC	%	0.18 (0.03)	0.12-0.26
Percentage of AMF colonization	AMFC	%	34.07 (14.82)	4.12-67.47

Table 4.2 Pearson correlation coefficients for pairwise traits with original data (lower-left diagonal). Correlations significant at $^{**}p < 0.01$, $^{*}p < 0.05$ are presented in bold.

	LT	SLA	LDMC	LCC	LNC	LPC	AD	SRL	RTD	RCC	RNC	RPC	AMFC
LT													
SLA	**0.26***												
LDMC	**0.34****	**0.34****											
LCC	**0.53****	**0.67****	**0.53****										
LNC	**0.34****	**0.63****	**0.30****	**0.76****									
LPC	**0.36****	**0.48****	**0.33****	**0.50****	**0.42****								
AD	-0.02	0.02	0.03	0.03	0.04	**0.22***							
SRL	0.15	0.07	0.03	0.01	-0.09	-0.09	**-0.79***						
RTD	-0.08	0.04	0.06	0.03	0.09	**0.22***	**0.58***	**-0.81****					
RCC	-0.05	0.00	0.02	-0.04	-0.20	-0.10	**-0.21***	**0.29****	-0.03				
RNC	-0.07	-0.07	-0.04	-0.10	**-0.24***	-0.11	**-0.32****	**0.43****	**-0.51****	**0.32****			
RPC	-0.02	0.09	0.00	0.11	0.03	0.07	0.02	0.06	-0.06	-0.01	**0.33****		
AMFC	0.03	-0.13	0.13	0.09	-0.04	-0.10	-0.11	0.09	-0.18	0.08	**0.32****	0.15	

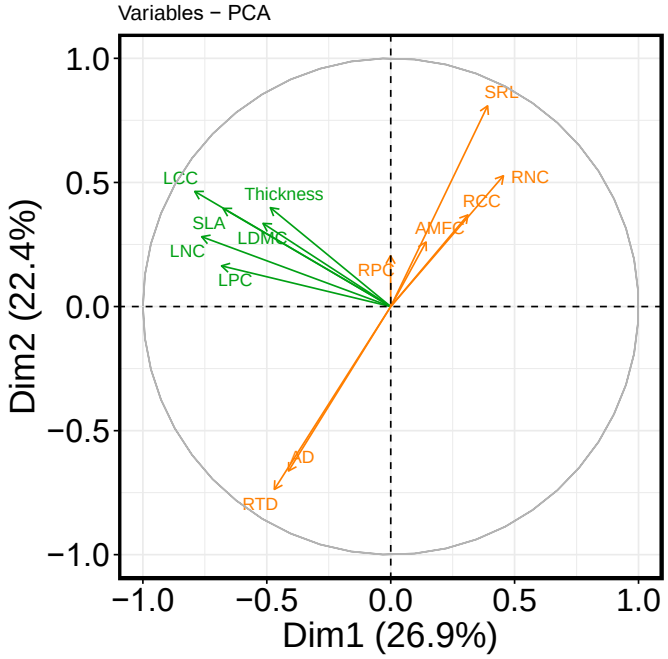


Figure 4.1 Principal component analyses (PCA) of plant community-weighted mean (CWM) traits under soil inoculation treatments. Arrows on the figure show the projections of the CWM traits within the PCA (green arrows indicate CWM leaf traits, orange arrows indicate CWM root traits).

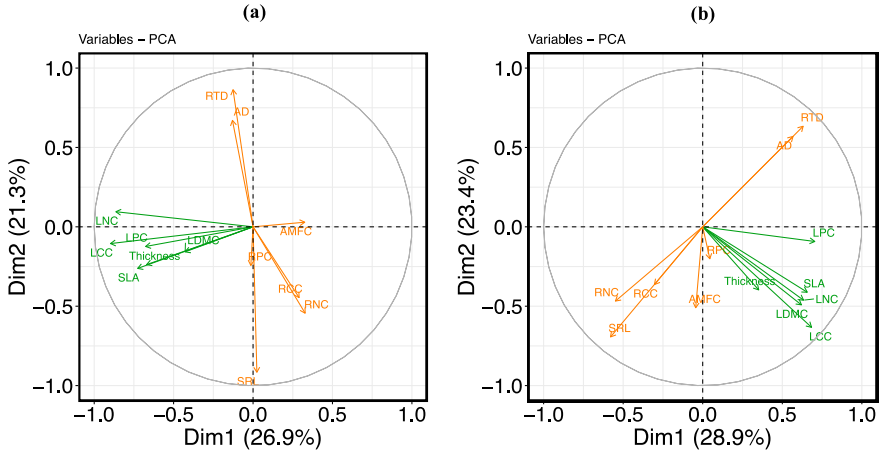


Figure 4.2 (**a**) Principal component analyses (PCA) of plant community-weighted mean (CWM) traits under (a) living soil inoculation and (**b**) sterile soil inoculation. Arrows on the figure show the projections of the CWM traits within the PCA (green arrows indicate CWM leaf traits, orange arrows indicate CWM root traits).

- *Impacts of soil inoculation treatments on the variation of aboveground vs belowground CWM traits*

The PERMANOVA revealed that the variation in plant CWM trait values was significantly influenced by soil inocula origin (pseudo-F = 2.23, $p < 0.05$, 7% explained variation, Table 4.3) and by the interactive effect of soil inocula origin and soil sterilization (pseudo-F = 2.02, $p < 0.05$, 6% explained variation, Table 4.3). Interestingly, when the CWM trait values were separated into leaf and root traits, leaf traits composition was only significantly affected by soil inocula origin (pseudo-F = 3.05, $p < 0.05$, 9% explained variation, Table 4.3). In contrast, root traits were significantly affected by the interactive influence of soil inocula origin and soil sterilization (pseudo-F = 3.33, $p < 0.05$, 10% explained variation, Table 4.3), suggesting that the added soil biota likely to play a more important role in modifying the values of root traits, compared to affecting leaf traits.

- *Response of individual CWM traits to soil inoculation treatments*

Community-weighted mean values of LDMC, LCC and LNC were significantly influenced by the addition of different types of soil inocula with soil sterilization having no effect (Table 4.4). This indicates that the effects were caused by the origin of the soil inocula and likely by the small amount of soil nutrients added with inoculation than by soil biota. The addition of soil inocula from later dune ecosystems (grasslands, forests) had a negative effect on LDMC (Figure 4.3c), LCC (Figure 4.3d) and LNC (Figure 4.3e) and values of these traits tended to be lower in plots with soil inocula compared to control plots that had no soil inocula. LCC tended to be lower in plots with soil inocula from dune grassland and dune forest (Figure 4.3d), while there was no difference in the response of LNC (Figure 4.3e) and LDMC (Figure 4.3c) to different types of soil inocula. The SLA also showed the same response pattern to the addition of later-successional soil inocula although these responses were non-significant (Figure 4.3b).

The responses of plant community roots traits were consistent with the patterns shown in the PCA plot. Some CWM root trait values were significantly influenced by the interactive effects of soil inocula origin and sterilization. For example, the effects of soil inocula on AD and SRL depend on the sterilization treatment of soil inocula (Table 4.4). Plant communities had thicker and denser roots when grown in plots with sterile grassland soil inocula than when grown in plots with living inocula (Figure 4.3g; Figure 4.3h). Changes in soil abiotic condition through soil inocula had significant influences on RPC and AMFC (Table 4.4). The addition of later-successional soil inocula had a negative effect on RPC leading to higher RPC values when grown in plots treated with dead soil inocula (Figure 4.3l). Plant communities had higher AMFC when grown in plots with dune soil inocula compared to plant communities grown in plots treated with grassland soil inocula (Figure 4.3m). In addition, there was a marginally significant (p=0.073) impact of soil sterilization on RNC with plant communities having higher RNC values in plots with living soil inocula (Table

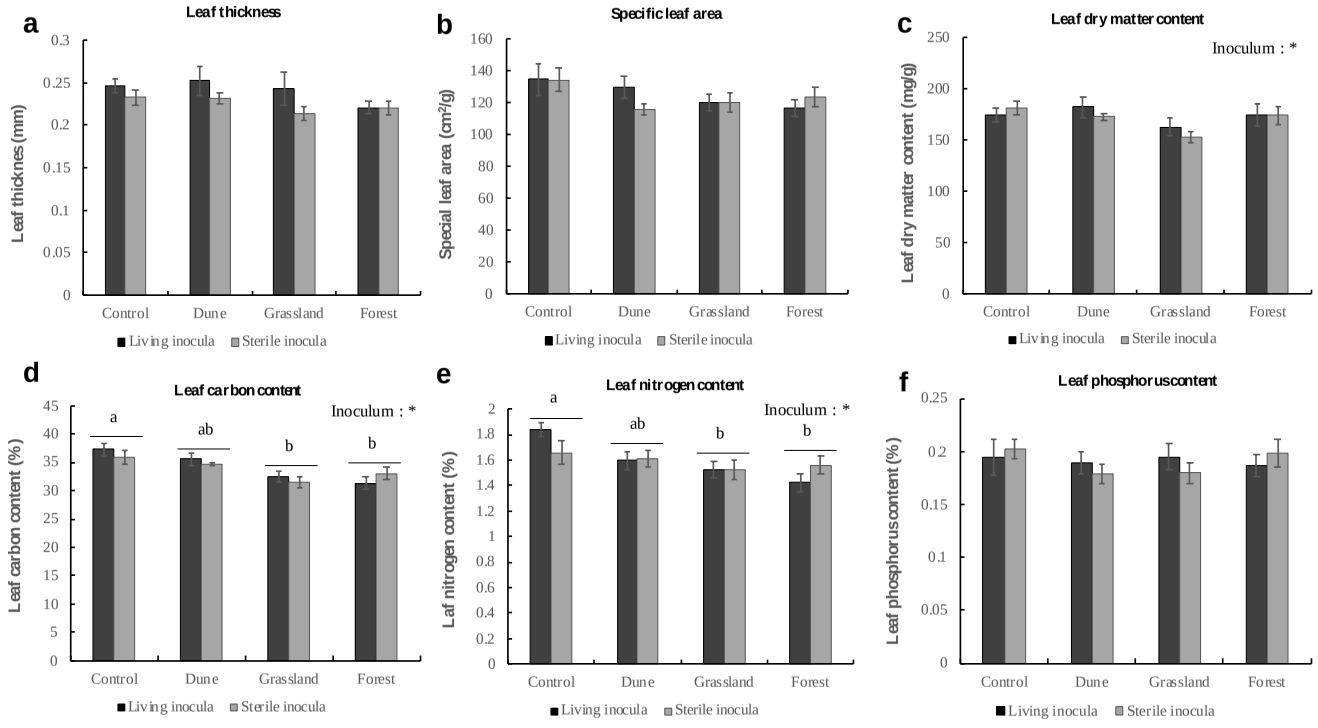
4.4; Figure 4.3k).

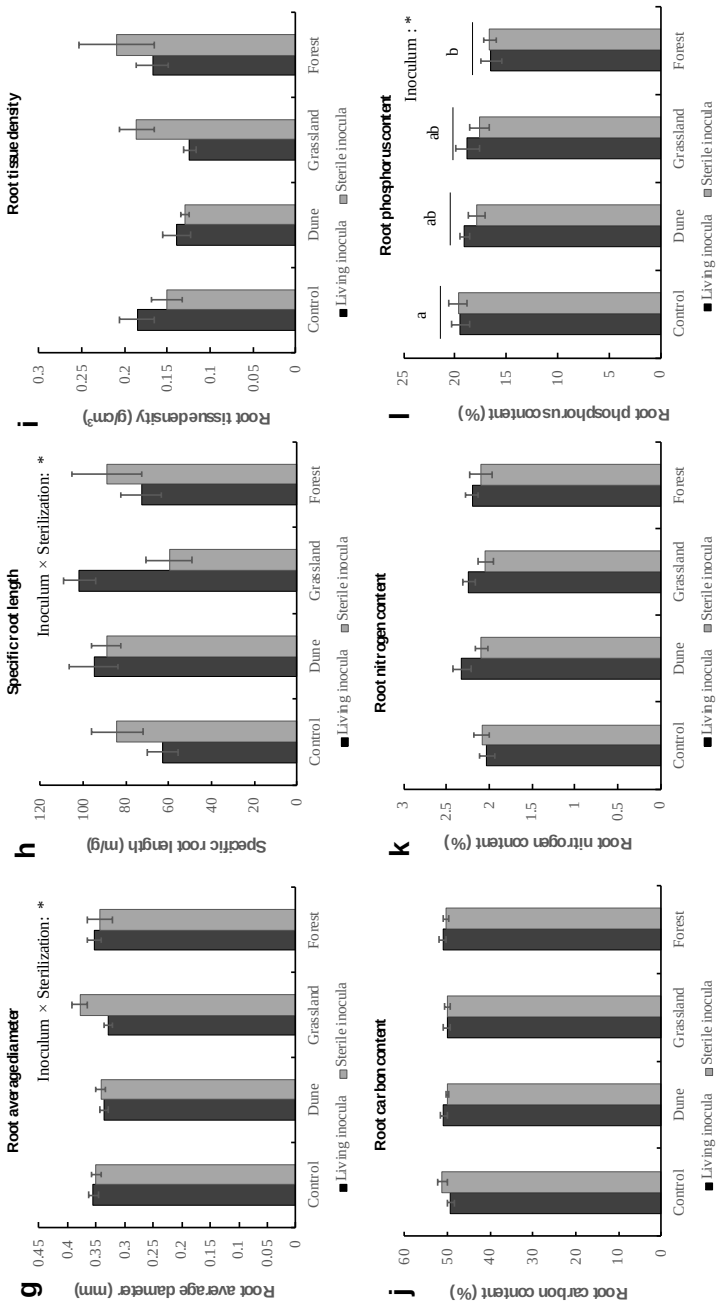
Table 4.3 Summary statistics of a PermANOVAs testing the effects of different types of soil inocula, soil sterilization and their interactions on the response patterns of CWM traits (Inoculum, I; Sterilization, S; permutation=9999). Presented are degrees of freedom, variance explained (R^2), F-values and p-values. Significant effects (p<0.05) are presented in bold.

CWM traits	Treatments	df1,df2	F-value	R^2	p-value
All traits	**Inoculum**	**3, 93**	**2.23**	**0.07**	**0.029**
	Sterilization	1, 93	0.42	<0.01	0.718
	I x S	**3, 93**	**2.02**	**0.06**	**0.048**
Leaf traits	**Inoculum**	**3, 93**	**3.05**	**0.09**	**0.013**
	Sterilization	1, 93	0.28	<0.01	0.743
	I x S	3, 93	0.43	0.01	0.830
Root traits	Inoculum	3, 93	1.25	0.04	0.283
	Sterilization	1, 93	0.50	<0.01	0.503
	I x S	**3, 93**	**3.33**	**0.10**	**0.018**

Table 4.4 Effects of different types of soil inocula (Inoculum, I), soil sterilization (Sterilization, S) and their interaction on the plant CWM traits (*F*, *F*-value; *p*, *p*-value; η^2, eta squared). Significant effects ($p<0.05$) are presented in bold.

	Source of variance									Transformation
	Inoculum			Sterilization			I × S			
Response	*F*	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2	
LT	1.90	.137	.059	3.50	.065	.036	0.41	.746	.013	log()
SLA	2.20	.094	.069	0.13	.722	.001	0.97	.412	.030	-
LDMC	**3.08**	**.032**	**.095**	0.45	.502	.005	0.51	.677	.016	-
LCC	**10.41**	**<0.01**	**.261**	0.32	.574	.003	1.11	.351	.028	-
LNC	**4.86**	**<0.01**	**.140**	0.01	.992	.001	1.60	.196	.046	-
LPC	0.57	.636	.019	0.04	.841	.001	0.65	.583	.022	-
AD	0.63	.600	.020	1.08	.302	.011	**2.80**	**.045**	**.087**	log()
SRL	1.03	.384	.031	0.12	.727	.001	**3.63**	**.016**	**.110**	-
RTD	1.71	.172	.052	0.40	.530	.004	2.39	.074	.073	log()
RCC	0.22	.882	.008	0.01	.997	.001	1.39	.252	.047	log()
RNC	0.88	.457	.029	3.29	.073	.036	0.95	.421	.031	-
RPC	**4.03**	**.010**	**.126**	0.64	.427	.007	0.39	.758	.012	-
AMFC	**2.96**	**.037**	**.093**	0.45	.504	.005	.544	.654	.017	-


a
Leaf thickness
Leaf thicknes (mm)
0.3
0.25
0.2
0.15
0.1
0.05
0
Control
Dune
Grassland
Forest
Living inocula
Sterile inocula
b
Specific leaf area
Special leaf area (cm²/g)
160
140
120
100
80
60
40
20
0
Control
Dune
Grassland
Forest
Living inocula
Sterile inocula
c
Leaf dry matter content
Inoculum : *
Leaf dry matter content (mg/g)
250
200
150
100
50
0
Control
Dune
Grassland
Forest
Living inocula
Sterile inocula
d
Leaf carbon content
Inoculum : *
Leaf carbon content (%)
45
40
35
30
25
20
15
10
5
0
a
ab
b
b
Control
Dune
Grassland
Forest
Living inocula
Sterile inocula
e
Leaf nitrogen content
Inoculum : *
Leaf nitrogen content (%)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
ab
b
b
Control
Dune
Grassland
Forest
Living inocula
Sterile inocula
f
Leaf phosphoruscontent
Leaf phosphoruscontent (%)
0.25
0.2
0.15
0.1
0.05
0
Control
Dune
Grassland
Forest
Living inocula
Sterile inocula


g
Root average diameter
Inoculum × Sterilization: *
Root average damater (mm)
h
Specific root length
Inoculum × Sterilization: *
Specific root length (m/g)
i
Root tissue density
Root tissuedensity (g/cm³)
j
Root carbon content
Root carbon content (%)
k
Root nitrogen content
Root nitrogen content (%)
l
Root phosphorus content
Inoculum : *
a
ab
ab
b
Root phosphorus content (%)
Control
Dune
Grassland
Forest
Living inocula
Sterile inocula

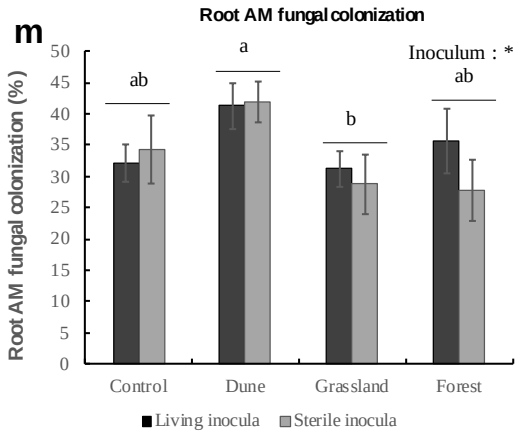


Figure 4.3 Effects of soil inoculation on community level mean weighted trait values: **(a)** LT; **(b)** SLA; **(c)** LDMC; **(d)** LCC; **(e)** LNC; **(f)** LPC; **(j)** RCC; **(h)** RNC; **(i)** RPC; **(j)** SRL; **(k)** AD; **(l)** RTD; **(m)** AMFC. *p<0.05, Data are means ± SE. The black bar indicate plots with living soil inocula, and the grey bar indicate plots with sterile soil inocula. Text with a star (*) indicated a factor revealed to be significant by the two-way ANOVAs. In case of the inocula origin being significant factor, letters above the bars indicate the outcomes of post-hoc analysis conducted to compare the impacts of distinct inocula origins.

4.4 Discussion

Our study demonstrates that community-level aboveground and belowground responses of plant traits to manipulated soil conditions are independent from each other. We further found that community-level leaf traits only depend on soil abiotic conditions, while root traits show variable responses to changed soil abiotic and biotic conditions. Yet, the differences between drivers of root and leaf traits do not obscure the orthogonality of these two trait groups.

Our study is unique in experimenting with impacts of sterilized versus living soil inocula on plant trait relations at field conditions. On the one hand, this allows explicitly accounting for the role of added ecologically realistic suits of soil organisms. On the other hand, it is important to realize that absence of a response to added soil biota (i.e. impact of the sterilization treatment and interactions therewith), does not necessary imply the absence of impact of soil biota per se. Plots treated with sterilized as well as non-sterilized inocula most certainly have been colonized by locally available soil organisms. Thus, trait responses to soil abiotic conditions, in combination with the absence of a response to soil biota additions, suggest a response to the activity of these local biota as mediated by the addition of sterilized inoculum. Collectively, in the follow up discussion, we explicitly articulate the effects of *experimentally added* soil inocula versus possible impacts of soil inocula in general, on for instance root functioning.

- *Community-level leaf and root traits orthogonality*

There was no significant correlation between community-level leaf and root traits, which strongly suggests that the response patterns of aboveground and belowground community-level traits to alternations in soil abiotic and biotic conditions were not coordinated. These results contrast the paradigm of coordinated leaf and root economic spectra at plant species level and are consistent with outcomes of some recent studies on the lack of coordination between root economic spectra and leaf spectra across individual species (Isaac *et al.* 2017; Wang *et al.* 2017). One explanation for the decoupled relationship of root and leaf traits lays in the large variety of root functions and resulting complex relationships to soil biotic and abiotic conditions, as proposed in the "multidimensional root trait framework" (Kramer-Walter *et al.* 2016; Weemstra *et al.* 2016). Compared to leaf traits which are mainly directly driven by the main function of light and CO_2 capture (Hendrik *et al.* 2009), the variation in root traits is driven by a more complex suit of abiotic and biotic selective pressures (Bardgett *et al.* 2014; Weemstra *et al.* 2016; Laliberté 2017). Such constraints to root traits do not directly operate in leaves, resulting in a larger variety of belowground resource acquisition mechanisms and trade-offs (Bardgett *et al.* 2014; Freschet *et al.* 2018). Therefore roots have more freedom to construct a variety of different trait combinations and may improve plant fitness under different conditions (Laughlin 2014).

Secondly, outsourcing the nutrient acquisition task to beneficial soil microbes, such as mycorrhizal fungi, could also reduce the necessity of developing a profound system of acquisitive roots. Thus multiple aspects of soil biotic and abiotic conditions, for which there are no analogues may control root traits (Ma *et al.* 2018; Bergmann *et al.* 2020). In agreement with this supposition, our results show that manipulation of the soil community influenced only the root traits but not leaf traits (Table 4.3). The main variation in leaf and root traits was explained by orthogonal axes of PCA, for which the great fraction of variation in leaf traits was explained by axes 1, while the main variation in root traits was explained by axes 2 and 3 (Table S4-1). The only exception to this rule was a weak correlation between root and leaf nitrogen concentrations (Table 4.2). This indicates that the general nutrient allocation rules governing the partitioning of nitrogen aboveground and belowground are still partly valid across plant communities while other aspects of root functional trait variation weaken such correlations.

Remarkably, the orthogonality between aboveground and belowground traits was preserved in plots with both sterilized and non-sterilized soil inocula (Figure 4.2), despite the significant response of individual belowground traits to additions of living soil biota (Table 4.3). This indicates that the observed independence of community-level aboveground and belowground responses is not driven by the experimental additions of soil biota, but is a principal feature of plant community behavior across gradients of nutrient availability and soil biota types. Together, this suggests a complementarity in variation of leaf and root

functions in response to altered soil conditions. The extent to which such above-belowground trait orthogonality is a characteristic of an early successional dune plant community, as in our experiment, or is a general feature of plant community ecology, needs further investigation.

- *Responses of community-level traits to manipulation in soil biotic and abiotic conditions*

We found that the responses of certain individual CWM leaf traits (e.g. LDMC, LCC, LNC and LPC) only depend on the origins of soil inocula, while soil sterilization, and therewith added soil biota, did not cause any effects. This finding is inconsistent with earlier proposals that there is a close link between aboveground traits and soil community at an individual plant species or functional group level (Orwin *et al.* 2010; Lau & Lennon 2011). Thus, the relationships between plant aboveground traits and soil communities measured at individual species or in monocultures are not necessarily representative for natural communities. This could be due to differential species responses to soil treatments (Table S4-2). For example, in later successional ecosystems, annuals and early-successional plant species are stronger negatively affected by plant-soil interactions due to the build-up of host-specific plant pathogens (Kardol *et al.* 2006; Kulmatiski *et al.* 2008). In contrast, the effects of plant-soil interactions on later-successional plant species appear to be more positive (Kardol *et al.* 2006) allowing for instance stronger plant benefit of AMF (Koziol *et al.* 2015).

Root traits showed more variable responses to changes in soil conditions compared to leaf traits, and responded to the origin of soil inocula only. Root morphological traits were determined by both biotic and abiotic factors of added soil inocula. We speculate that in our system the presence of added soil biota supported nutrient acquisition by influencing the root morphological traits (higher SRL, lower AD and RTD). These results highlight that manipulations of soil biota alter the root architecture of plant community. This is consistent with previous studies which have shown that biotic interactions of roots with soil biota influence the architectural, morphological root traits across plant species (Vacheron *et al.* 2013; Bergmann *et al.* 2020; Grover *et al.* 2021). Moreover, we found that the presence of added soil biota may promote a "fast-growing" plant community characterized by a resource-exploitative strategy (construction of long, narrow-diameter roots with minimal biomass investment but high metabolic rates) (Ryser & Eek 2000; Ostonen *et al.* 2007; Reich 2014). This may be explained by the positive effects of accumulated beneficial soil community from later-successional donor ecosystems, such as plant growth-promoting bacteria and rhizobia. These beneficial communities can increase root length and result in increased plant growth and development (Vacheron *et al.* 2013; Grover *et al.* 2021).

Plant communities had a lower root AM fungal colonization when grown with later-successional soil inocula, suggesting that the plant community invests less in its association with AM fungi when grown in better nutrient conditions. Alternatively, these results may be

explained by the fact that our experiment was conducted in an early development stage in which most dominant species were early-successional plants (Table S3-1) which may depend less on AM fungal symbiosis for nutrient uptake (Middleton & Bever 2012; Koziol *et al.* 2015).

Unlike other root traits, RPC was lower in plant communities grown in plots with late-successional soil inocula, in comparison with other inoculation treatments. This may have been caused by the extremely low values of soil P in our system (van der Heijden, 2010). In such situation, uptake of P from fertile soil inocula may become the dominant P uptake pathway. The addition of soil nutrients from later-successional systems may then reduce the immobilization of P in soil and consequently influence the plant community P uptake efficiency (van der Heijden, 2010).

4.5 Conclusions

Our results provide the valuable evidence that the variation in plant community-level leaf traits across soil conditions are not correlated to the variation in community-level root traits. Community-level leaf traits (LDMC, LCC and LNC) were mainly determined by the changes in the soil abiotic conditions. On the other hand, root traits showed higher variation along shifts in both soil abiotic and biotic conditions. The plant communities tended to have more nutrient-acquisitive root traits (high SRL, low AD and RTD) in the presence of added soil biota. We conclude that soil inoculations with soil communities affect ecosystem functioning through the modification of belowground, but not aboveground, community-weighted mean values of plant traits. Our study sets a benchmark in explicit and evidence-based understanding of the role of soil biotic and abiotic conditions in ecosystem functioning. As a next step, we encourage further efforts to test the general pattern of leaf and root correlations across different ecosystems under field conditions.

4.6 Acknowledgements

We are grateful to Dunea Duin & Water company for the help with establishment of the experiment. Funding was provided by the Netherlands Organization for Scientific research (NWO; VIDI Grant No. 016.161.318 issued to NAS; VICI grant 865.14.006 issued to TMB) and the China Scholarship Council (Grant No. 201804910632) issued to C.G.

4.7 Author contributions

CG, NAS and PMB design the research. NAS, TMB, RM and HH established the experiment of TERRA-Dunes. CG, XL and RM collected samples. CG, RL, MZ processed the samples in lab. CG analyzed the data with helpful input from NAS, PMB, TMB, HCC and XL. CG wrote the first draft, and all authors contributed to editing the manuscript.

4.8 Supporting information

Table S4-1 Results of the principal component analysis on the plant CWM trait values, including the proportion of variation explained.

Component	Eigenvalue	Proportion (%)	Cumulative (%)
1	3.50	0.27	0.27
2	2.90	0.22	0.49
3	1.33	0.10	0.60
4	1.02	0.08	0.67
5	0.92	0.07	0.74
6	0.78	0.06	0.80
Variable	Component 1	Component 2	Component 3
LT	-0.26	0.23	-0.10
SLA	-0.36	0.23	-0.06
LDMC	-0.28	0.20	0.11
LCC	-0.42	0.27	0.06
LNC	-0.41	0.16	-0.09
LPC	-0.36	0.10	0.07
AD	-0.22	-0.39	0.29
SRL	0.21	0.47	-0.25
RTD	-0.25	-0.43	0.11
RCC	0.17	0.22	0.03
RNC	0.24	0.31	0.40
RPC	-0.01	0.12	0.60
AMFC	0.08	0.15	0.53

Table S4-2 Effects of different types of soil inocula (Inoculum, I), soil sterilization (Sterilization, S) and their interaction on traits of individual plant species (F, F-value; p, p-value). Significant effects ($p<0.05$) are presented in bold.

Species	Traits	Variance					
		Inoculum		Sterilization		I × S	
		F	*P*	*F*	*P*	*F*	*P*
Plantago lanceolata	LT	0.93	0.43	2.74	0.10	0.41	0.75
	SLA	1.03	0.38	0.53	0.47	0.51	0.67
	LDMC	1.98	0.12	1.64	0.20	0.49	0.69
	LCC	0.95	0.42	2.68	0.11	1.34	0.27
	LNC	0.14	0.93	0.19	0.66	1.45	0.23
	LPC	2.02	0.12	0.13	0.72	**3.01**	**0.03**
Elytrigia repens	LT	0.76	0.52	0.27	0.61	0.64	0.59
	SLA	1.37	0.26	0.89	0.35	0.10	0.96
	LDMC	0.74	0.53	1.17	0.28	0.73	0.54
	LCC	0.34	0.79	0.23	0.63	**3.20**	**0.03**
	LNC	0.08	0.97	0.42	0.24	0.57	0.64
	LPC	0.62	0.60	**5.18**	**0.03**	0.92	0.44
Crepis capillaris	LT	0.79	0.50	1.63	0.21	1.41	0.25
	SLA	0.05	0.99	**5.48**	**0.02**	1.23	0.31
	LDMC	0.78	0.51	1.06	0.31	0.83	0.48
	LCC	1.20	0.32	2.53	0.12	1.21	0.31
	LNC	1.54	0.21	1.45	0.23	0.30	0.83
	LPC	1.99	0.12	1.06	0.31	0.14	0.93
Senecio inaequidens	LT	1.76	0.16	0.29	0.59	0.83	0.48
	SLA	0.25	0.86	1.06	0.31	1.99	0.13
	LDMC	0.55	0.65	1.18	0.28	0.26	0.86
	LCC	1.05	0.38	0.95	0.33	2.20	0.10
	LNC	0.39	0.76	0.01	0.95	**4.03**	**0.01**
	LPC	2.07	0.11	0.15	0.70	0.56	0.64
Oenothera spec.	LT	**2.88**	**0.05**	0.09	0.77	0.42	0.74
	SLA	1.07	0.37	1.37	0.25	2.46	0.08
	LDMC	0.22	0.88	0.16	0.69	**4.88**	**0.01**
	LCC	1.18	0.33	0.27	0.61	0.13	0.94

Species	Traits	Variance					
		Inoculum		Sterilization		I × S	
		F	*P*	*F*	*P*	*F*	*P*
Oenothera spec.	LNC	0.62	0.61	0.04	0.85	1.43	0.25
	LPC	1.03	0.39	0.31	0.58	0.75	0.53
Conyza canadensis	LT	0.35	0.79	0.11	0.74	0.73	0.54
	SLA	0.32	0.81	0.59	0.45	0.25	0.86
	LDMC	1.85	0.17	0.00	1.00	1.28	0.30
	LCC	0.49	0.69	0.17	0.68	0.21	0.89
	LNC	0.88	0.46	0.02	0.89	0.25	0.86
	LPC	0.11	0.95	0.11	0.75	0.57	0.64
Anthyllis vulneraria	LT	2.12	0.11	0.32	0.57	1.89	0.16
	SLA	2.25	0.09	0.02	0.89	1.06	0.35
	LDMC	0.87	0.46	0.33	0.57	0.84	0.48
	LCC	0.70	0.55	0.72	0.40	0.98	0.38
	LNC	2.18	0.10	0.21	0.65	2.53	0.09
	LPC	1.40	0.25	0.83	0.37	0.08	0.92
Helictotrichon pubescens	LT	0.09	0.96	0.14	0.71	2.63	0.06
	SLA	0.94	0.42	3.14	0.08	0.14	0.93
	LDMC	0.87	0.46	0.33	0.57	0.84	0.48
	LCC	0.95	0.42	0.74	0.39	2.53	0.06
	LNC	0.71	0.55	3.56	0.06	0.24	0.87
	LPC	2.06	0.11	**4.46**	**0.04**	1.54	0.21
Daucus carota	LT	0.77	0.52	0.01	0.99	0.47	0.70
	SLA	0.77	0.51	0.01	0.99	1.19	0.32
	LDMC	1.78	0.16	2.04	0.16	1.53	0.22
	LCC	0.14	0.94	2.69	0.11	0.47	0.70
	LNC	0.83	0.48	0.16	0.69	0.64	0.59
	LPC	0.03	0.99	2.09	0.15	0.62	0.61