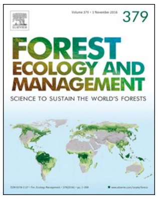
Forest Ecology and Management 479 (2021) 118605

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco



Functional traits of individual varieties as determinants of growth and nitrogen use patterns in mixed stands of willow (*Salix* spp.)

Martin Weih[a,*], Nils-Erik Nordh[a], Stefano Manzoni[b], Stefanie Hoeber[a]

[a] *Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden*
[b] *Department of Physical Geography, Stockholm University, Stockholm, Sweden*

ARTICLE INFO

Keywords:
Biomass production
Functional traits
Growth analysis
Nitrogen use efficiency
Short rotation forestry
Variety mixtures

ABSTRACT

Short rotation plantations of willows (*Salix* spp.) have high biomass production potential in many parts of the world, and may frequently support ecosystem services related to nutrient cycling. A plantation management enhancing favorable environmental impacts that are conducive to maintaining ecosystem services is a main challenge in establishing sustainable biomass production systems. There is evidence supporting the hypothesis that biomass production and nutrient cycling can be increased by supporting ecosystem niche differentiation (complementarity) through enhancing the number of plant species or varieties grown in the stand. However, the specific trait values of the individual components (e.g., varieties) in a mixed community could also be more important than the community diversity *per se*. We assessed, at community level, the plant trait profiles related to growth and nitrogen (N) use for four different *Salix* varieties that were taxonomically distinct at species or genotype level ('Björn', 'Jorr', 'Loden', 'Tora') and field-grown in unfertilized plots of pure and mixed communities during one cutting cycle in Central Sweden. The aims were to use elements of functional growth analysis for exploring the mechanistic relationships between various traits related to growth and N use at stand level in our pure and mixed willow communities; and to address two hypotheses related to (i) the effect of diversity level on above-ground traits linked to growth, N uptake efficiency, N productivity and N conservation; and (ii) the influence of individual variety identities on the growth and N use traits observed in a mixture. Diversity level had no significant effect on the traits assessed here, and we thus found no evidence in support of our hypothesis that traits linked to growth, N uptake and use are significantly affected by the diversity level *per se*. In most but not all cases, the admixing effects on trait values were explained by the effects of the individual variety characteristics assessed in monocultures in combination with their relative share in the respective mixtures. The absence or presence of individual varieties strongly affected community-averaged (stand level) trait values. Therefore, the design of desirable variety mixtures is suggested that combine, for example, the high nutrient conversion efficiency that certain varieties achieve in mixed stands with the specific nutrient acquisition characteristics of other varieties.

1. Introduction

Intensively managed plantations of willows (genus *Salix*, family Salicaceae) are grown on agricultural land especially in temperate and arctic zones of the northern hemisphere (Dickmann and Kuzovkina, 2014). In particular when grown in short rotation, these trees or shrubs are gaining increasing interest worldwide because of their efficient and sustainable land use (Karp and Shield, 2008; Weih et al., 2020), and they are also suitable model systems for exploring ecological theory on biodiversity-ecosystem function (Weih et al., 2019). An important issue in sustainable land use for biomass production is the goal to reduce the depletion of nutrient resources (Higman et al., 2005; Ra et al., 2012); whilst at the same time, the efficient uptake and use of mineral nutrients, in particular nitrogen (N), is a critical management factor in willow production. Thus, nutrient fertilizers are frequently used to increase willow productivity, and selection and breeding progress for increasing productivity of biomass willows often rely on the great variability of varieties in regard to their responses to increased supply of nutrient fertilizer (Weih and Nordh, 2005; Fabio and Smart, 2018a). However, higher productivity accomplished by greater nutrient (e.g., N) supply is frequently associated with the use of synthetic fertilizers. According to life cycle assessment, the production and use of synthetic fertilizers contributes most of all management actions to the energy use in willow plantations (Hammar et al., 2017) and also increases the

---

[*] Corresponding author at: Department of Crop Production Ecology, Swedish University of Agricultural Sciences, PO Box 7043, SE-750 07 Uppsala, Sweden.
E-mail address: martin.weih@slu.se (M. Weih).

https://doi.org/10.1016/j.foreco.2020.118605
Received 5 June 2020; Received in revised form 7 September 2020; Accepted 8 September 2020

Nomenclature

E_N	Yield-specific nitrogen (N) efficiency (shoot increment per growing-season mean plant N pool, kg (kg N)$^{-1}$ yr^{-1})
LAI*	Leaf area index (leaf area per ground area, m^2 m^{-2})
LAP	Leaf area productivity (shoot biomass increment per leaf area, g m^{-2} yr^{-1})
LAR	Leaf area ratio (leaf area per plant biomass, m^2 kg^{-1})
LBR	Leaf biomass ratio (leaf biomass per plant biomass, kg kg^{-1})
LNC*	Leaf N concentration (g N per g leaf biomass)
LNLA*	Leaf N content per leaf area (mmol N m^{-2})
N_s	Initial plant N pool in the perennial biomass (kg N plot^{-1})
N'	Mean plant N pool during the main growing season (kg N plot^{-1})
NAE	N accumulation efficiency (final shoot N pool per initial shoot N pool, kg N (kg N)$^{-1}$ yr^{-1})
PNC	Mean plant N concentration during growing season (g N per g above ground plant biomass)
R_{SB}	Relative shoot biomass annual increment (annual increment per initial shoot biomass, yr^{-1})
SB	Shoot biomass annual increment (kg plot^{-1} yr^{-1})
SLA*	Specific leaf area (leaf area per leaf biomass, m^2 kg^{-1})
SNC*	Shoot N concentration (g N per g shoot biomass)
U_N	N uptake efficiency (N' per N_s, kg N (kg N)$^{-1}$)
*	Subscripts indicating sampling occasions e.g. in (early) October 2016 (peak season) respective March 2017 (shoot harvest)

carbon footprint (Lutes et al., 2019), overall leading to less sustainable land use (Higman et al., 2005). In addition, while nutrient fertilization greatly enhances biomass yields, it usually decreases the productivity per unit of plant-internal amount of N (Weih et al., 2018), reflecting the general pattern of more than proportional resource demand, or diminishing returns, when yields of crops are increased through fertilization (Tilman et al., 2002). Therefore, in willow plantations, sustainability oriented approaches could focus on management actions, including the choice of plant material and plantation design, that increase productivity while maintaining (or decreasing) nutrient uptake and depletion from the soil. For example, growing more diverse willow stands has been discussed as a means to increase nutrient resource complementarity and nutrient use efficiency, but so far, no clear evidence has been documented for a strong positive effect of stand diversity on productivity in young willow plantations (Dillen et al., 2016; Hoeber et al., 2018). Although the characteristics of specific willow varieties have been shown to affect stand productivity, as indicated by the significant effects that the presence of certain varieties can have on stand productivity, little is known about the mechanistic relationships linking the underlying traits to productivity (Hoeber et al., 2018).

In general, plant functional traits are important for explaining plant productivity, and they have been used to relate the responses of plant taxonomical entities (such as species and varieties) to the environmental conditions; or to mechanistically link the specific influences of plants on ecosystem processes (Lavorel and Garnier, 2002). In addition to the strong influence of genotype (e.g., species, varieties), functional traits are plastic and thereby contribute to acclimation of plant communities to their environments; their plasticity is thus expected to increase complementarity and thereby modulate plant-plant interactions (Perez-Ramos et al., 2019). Consequently, certain trait values observed in a mixed community can be expected to differ from the values calculated from their measurements on the same community components grown in monoculture. As a result, those traits contribute to the frequently observed increase in biomass production with enhanced diversity through complementarity (Tilman et al., 1997; Loreau and Hector, 2001). However, there is a lack of a clear understanding of the mechanistic interaction between the traits underpinning the observed patterns in diversity-productivity relationships (Turnbull et al., 2013), and the results obtained are also hard to generalize due to conflicting interpretations of the same results (Schulze et al. 2018). By focusing on taxonomic predictors rather than the trait profiles of the investigated species or genotypes, investigators have more often studied the consequences of community assemblages (in terms of taxonomic entities) for plant growth and productivity, and not the functional mechanisms behind observed patterns. Taxonomic predictors are occasionally replaced by those based on the traits of the involved community components, but this approach is challenging because functional traits may influence processes at different levels of organization (Holzwarth et al., 2015) and the trait influence on these processes is modulated by both their genetic background and associated phenotypic plasticity. In addition, traits may be assessed at different levels of organization within a community (Garnier et al., 2004; Shipley et al., 2006); and the traits assessed at higher level (e.g. including several organs) are likely to show greater plasticity, due to greater complementarity of multiple less-aggregated traits, than those assessed at lower level (e.g. single organs such as the properties of single leaves) (Siefert et al., 2015). Functional growth analysis (Hunt, 1982) offers an approach in which traits at both high and low levels of organization are linked to overall growth and productivity, because relative growth rate (RGR) is here hierarchically decomposed into traits at higher level (e.g., leaf area ratio, LAR) and lower level (e.g., specific leaf area, SLA) (Lambers et al., 1990). Functional growth analysis is therefore a suitable tool to explore hypotheses related to the mechanistic links between functional traits assessed at different organization levels and in different diversity contexts.

In pot-grown willows, we have established links between traits related to growth and N economy by using elements of growth analysis (Weih, 2001; Weih and Nordh, 2002; Weih et al., 2011b; Hoeber et al., 2017); and we have also taken some first steps to explore relationships between growth and N use traits at stand level (Weih and Nordh, 2005; Weih and Ronnberg-Wastljung, 2007). One of the indications from this previous work is that, in the willows used here, variation in shoot growth rate is driven less by variation in biomass allocation (e.g., LAR; SLA) and more by variation in N uptake and allocation (Weih, 2001; Weih and Nordh, 2005; Weih et al., 2011b). Further, we evaluated the productivity of various willow varieties and mixtures of different diversity levels for the first cutting cycle (Hoeber et al., 2018), but we were unable to link those results to the underlying traits because productivity was integrated over several years and no detailed data on individual growth and N use traits were available for all years. Functional growth analysis (Hunt, 1982) is challenging to apply at stand level, due to practical issues related to the representative sampling of traits at stand level, and difficulties to conform to basic principles such as the assumptions of exponential growth and steady-state growth (Ingestad and Agren, 1995). It is however possible to consider important elements of functional growth analysis at the stand level in tree plantations, by adopting the basic mechanistic relationships utilized by this approach. We use here both traits assessed at low organization level, such as SLA and leaf N concentrations; and traits at high level, including LAR, leaf area productivity (LAP) and the traits reflecting stand-level N uptake and use (Weih et al., 2011a).

The overall aim was to use elements of functional growth analysis for exploring the mechanistic relationships between various traits related to growth and N use at community level in a willow plantation of four contrasting varieties grown in plots of pure and mixed cultures. The field trial was established in 2014 and is part of the TreeDivNet network (www.treedivnet.ugent.be), which is the largest network of biodiversity experiments worldwide (Verheyen et al., 2016). Assuming that community components (here varieties) differ in their functional traits assessed both at high and low levels of organization, we explored the following hypotheses: *(H1)* Growth and N use traits are significantly affected by the diversity level, especially for traits at high level of organization for which the effects of trait plasticity are compounded. *(H2)* The presence or absence of individual varieties in a mixture significantly affects the growth and N use traits observed in a mixture; as a consequence, there are variety traits for which the mean values observed in a mixture are different from the weighted average values calculated from community components grown in pure cultures.

2. Material and methods

2.1. Field trial

The field trial was established on arable land in Uppsala, Central Sweden (59° 49′ N, 17° 39′ E) in May 2014. This trial is part of a larger global tree diversity network (TreeDivNet), which aims at exploring the relationships between tree species or genotype diversity and ecosystem function (Verheyen et al., 2016). Cuttings of four different *Salix* varieties, taxonomically distinct at species or genotype level, were planted: 'Björn' (*Salix schwerinii* E. Wolf. × *S. viminalis* L.) and 'Tora' (*S. schwerinii* × *S. viminalis*), the two are full-siblings; 'Jorr' (*S. viminalis*) and 'Loden' (*S. dasyclados* Wimm.), the latter most distinct in terms of taxonomy from the other three varieties. The design was a randomized block design (three replicate blocks) of all monocultures and mixtures of the four genotypes (two, three and four mixtures), resulting in a total number of 45 plots. The two-genotype plots were planted in a checkerboard pattern according to a design created with a computer program; while planting positions were randomized in the three- and four-genotype plots, with the constraint of individuals belonging to the same genotype not being directly adjacent to each other within rows. Each plot was 9.6 m × 9.6 m in size and contained 12 rows with 12 plants per row, with an offset every second row, resulting in a hexagonal planting pattern with equal distances of between 0.8 and 0.9 m between individuals. The design corresponded to a planting density of approximately 15,600 plants ha^{-1}; the survival after 3 years of growth was estimated at 97% (Hoeber et al., 2018) and competitive losses (e.g. due to shading) were therefore assumed negligible during the investigation period considered here. No fertilizer was applied. Details of the planting procedure, plantation design and management are documented elsewhere (Hoeber et al., 2018). Soil characteristics in the field trial at its establishment, and weather conditions during the here relevant growing season (March to October 2016) are presented in Table 1, together with the corresponding 30-year means to illustrate the growing-season climate conditions in the area of this field trial. According to these data, the 2016 growing season was slightly warmer and drier than the current standard 30-year period (1961 to 1990).

2.2. Shoot biomass sampling

Shoot biomass was sampled from the 40 most central plants within each plot (sampling area 8.0 m × 3.2 m) before bud break (i.e., in March 2017) after the final year of the first 3-year cutting cycle; for details of the sampling procedure see Hoeber et al. (2018). To assess total leaf area, specific leaf area (SLA) and tissue N concentrations of individual plant parts (except roots) for the previous growing season, shoots and leaves of 12 randomly selected plants per plot were sampled in early October 2016 (before leaf abscission) from the subplots outside the sampling area assigned to the March 2017 sampling. Ten leaves were randomly collected from leader shoots (including the whole leafy range along the shoot) of the sample plants for determination of SLA and leaf N contents. To get an estimate of the annual shoot growth during the 2016 growing season, non-destructive assessments of shoot biomass were performed already in April 2016 on the same plants that later (in March 2017) were sampled destructively; shoot biomass (in April 2016) was then estimated based on variety-specific allometric relationships between shoot diameter and biomass (Verwijst and Telenius, 1999). Three 2-cm woody shoot sections were taken from all the shoots sampled in October 2016 and March 2017 for N analysis. Leaf area of fresh leaves was determined with a flatbed scanner and using ordinary software for area determination. All harvested plant parts were dried at 70 °C for 48 h, weighed and ground to pass a 1-mm mesh.

2.3. Assessments of leaf area index

Leaf area index (LAI, m^2 m^{-2}) was assessed non-destructively using a CI-110 Plant Canopy Imager (CID Bio-Science Inc., Camas, WA, USA) placed in the center of each plot on 12 July and 15 August 2016 to monitor leaf canopy development during the main growing season.

2.4. Chemical analysis of plant material

Total N contents of different plant fractions were analyzed after dry combustion (Dumas principle, ISO 13878) after pooling of samples from each plot (experimental unit).

2.5. Calculations of growth, nitrogen uptake and utilization efficiency

We used time-integrated means of community-level biomass and N pools to calculate various traits related to growth, N uptake and utilization efficiency. Thus, monthly means of shoot and leaf biomass were interpolated for the entire period between April 2016 and March 2017, based on the shoot biomass estimates for April 2016, the LAI measurements in July and August 2016, the shoot biomass and leaf area assessments in early October 2016, and the shoot biomass sampling in March 2017. The LAI measurements were used for estimating leaf areas in July and August by assuming similar LAI in August and early October, which was verified by additional sporadic LAI measurements (in 5 randomly chosen plots) performed between the end of August and early October. Based on previous data assessed in willow stands (Weih and Nordh, 2005; Weih and Ronnberg-Wastljung, 2007), the following coefficients were applied to estimate the annual shoot biomass increase between the measured figures in April and October 2016: 0.1 (May), 0.25 (June), 0.5 (July), 0.75 (August), and 0.9 (September) of the annual shoot growth achieved. The corresponding values for leaf area development were 0.3 (May), 0.5 (June) and 1.0 (September) of the maximum leaf area; leaf areas in July and August were estimated from the measured LAI as described above. The monthly biomass means were calculated as plot means for all 45 plots, and multiplied with the corresponding tissue N concentrations to also obtain monthly means for N

Table 1
The soil characteristics (0–10 cm depth) before planting, the climatic conditions as illustrated by the 30-year annual (growing season) means of air temperature and precipitation, and the corresponding means for the growing season between March and October 2016 as recorded nearby (< 1 km distance) the ECOLINK-Salix field trial in Uppsala*

Soil or weather characteristic	Quality or value
Soil type	Vertic Cambisol
Soil organic matter (%)	2.67
N_{total} (%)	0.17
C/N ratio	8.9
$pH_{(CaCl2)}$	6.2
Mean annual precipitation sum (mm) March to October 1961–1990	376
Mean annual temperature (°C) March to October 1961–1990	9.6
Mean annual precipitation sum (mm) March to October 2016	290
Mean annual temperature (°C) March to October 2016	11.2

* Weather and climate data are from the SLU Ultuna climate station, and soil data are from Hoeber et al. (2018)

pools for all 45 plots (Supplement S1). The monthly means (biomass and N pools) were used to calculate various traits related to community-level growth, N uptake, N productivity and N conservation in the stand (plot basis).

In order to explore (e.g., by means of correlation analysis) our hypotheses addressing the mechanistic links between growth and the underlying functional traits assessed on four varieties grown in different diversity contexts, we adopted elements of functional growth analysis (Hunt, 1982). This approach is suitable to track the mechanistic relationships driving growth and N use but, in its strict application, unfeasible to apply to trees grown at stand level in most cases, not least due to the frequent violation of basic principles such as the assumption of exponential growth rate (which only applies to juvenile plants) and steady-state growth (Ingestad and Agren, 1995). Thus, by calculating all traits independently and following conceptually their inherent links considered in functional growth analysis, we related relative shoot biomass increment during the 2016 growing season per initial shoot biomass at start of the growing season (R_{SB}; kg kg^{-1} yr^{-1}; or yr^{-1}) to stand-level mean leaf area ratio (leaf area per plant biomass, LAR; m^2 kg^{-1}) and leaf area productivity (shoot biomass increment per leaf area, LAP; g m^{-2} yr^{-1}, similar to the net assimilation rate by Lambers et al. (1990)):

$R_{SB} \approx LAR \times LAP$ (eq. 1)

where all components in the equation are at high level of organization (here the symbol ≈ indicates proportionality, allowing us to simplify the notation by avoiding unit conversion factors).

Following functional growth analysis, the LAR can be further decomposed into leaf biomass ratio (LBR; kg kg^{-1}) and specific leaf area (SLA; m^2 kg^{-1}); both of them are traits at a low level of organization but were still averaged across the individuals in a plot:

$LAR = LBR \times SLA$ (eq. 2)

By linking biomass production, leaf area and leaf N, the LAP can be decomposed further into the area-based leaf N content (LNLA; mmol N m^{-2}) and the biomass production rate per leaf N, or leaf N productivity (Lambers et al., 1990); the latter corresponds here to the annual shoot biomass increment per mean plant N pool (yield-specific N efficiency, E_N; kg (kg N)$^{-1}$ yr^{-1}) (Weih et al., 2011a) because, during the growing season, the majority of plant N is allocated to the leaves in willows:

$LAP \approx LNLA \times E_N$ (eq. 3)

where the E_N is a trait at high level of organization whilst the LNLA is at low level of organization.

Annual relative shoot increment was also directly linked to the mean above ground plant N concentration during the growing season (PNC; g N per g biomass), *sensu* Lambers et al. (1990):

$R_{SB} \approx E_N \times PNC$ (eq. 4)

Finally, N uptake and use were analysed using the concept of N accumulation efficiency (NAE), with “yield” represented by the harvested shoots (without leaves) and calculated on an annual basis. The NAE is the final shoot N pool per initial shoot N pool (kg N (kg N)$^{-1}$ yr^{-1}), and when calculated on an annual basis, it reflects the R_{SB} provided that the shoot N concentration (SNC; g N per g biomass) is considered similar at the start and end of the 12-month period, which was assumed here. The NAE is then decomposed into its components N uptake efficiency (U_N; kg N (kg N)$^{-1}$), yield specific N efficiency (E_N; kg (kg N)$^{-1}$ yr^{-1}) and yield N concentration which here is the SNC at shoot harvest in March 2017 (SNC_{Mar}); according to Weih et al. (2011a) and Weih et al. (2018). In this study, NAE and its components are based on the above ground plant parts (i.e., leaves and shoots) and N pools:

$NAE = U_N \times E_N \times SNC_{Mar}$ (eq. 5)

Following Weih et al. (2011a), U_N is the ratio between the mean plant N pool during the main growing season (N’; kg N plot^{-1}) and the N amount in the perennial biomass prior to the start of the growing season (N_s; kg N plot^{-1}) in April 2016; and E_N is estimated as the ratio between the harvested shoot biomass and N’.

All traits except LAI were assessed at variety level in all investigated mixtures, enabling us to evaluate variety-level trait variability by comparing the trait values measured when a given variety was grown in pure culture or admixed with other varieties.

2.6. Statistical analysis

Relationships among growth traits were evaluated by correlation (Pearson) and regression analyses as well as Student’s T-tests. One-way analysis of variance (GLM, ANOVA) was used to assess the effect of variety (fixed factor) on various community (plot level) response variables, using the data from the monoculture plots (n = 3) or individual mixture comparisons (e.g., mixtures of ‘Tora’ and ‘Loden’ *vs.* those of ‘Tora’ and ‘Björn’). Two-way ANOVA was used to assess the effects of diversity level (fixed factor; pure culture and plots with two, three and four varieties) and block (random factor) on community response

Table 2
Means ± SD of shoot growth and various functional traits[§] assessed in plots of pure cultures of four *Salix* varieties (‘Björn’, ‘Tora’, ‘Jorr’, ‘Loden’) field-grown during one growing season in Central Sweden. Significant differences between varieties (P_{ANOVA}) is indicated for one-way ANOVA, n = 3 (plots) (P ≤ 0.050 in bold).

	’Björn’	’Tora’	’Jorr’	’Loden’	P_{ANOVA}
SB (kg plot^{-1} yr^{-1})	41.5 ± 16.5	38.6 ± 5.1	30.9 ± 11.5	27.8 ± 12.4	0.508
R_{SB} (yr^{-1})	2.88 ± 0.37	3.44 ± 1.09	3.65 ± 1.64	3.18 ± 0.45	0.809
LAR (m^2 kg^{-1})	1.85 ± 0.26	1.83 ± 0.05	2.23 ± 0.34	2.56 ± 0.18	**0.016***
LAP (g m^{-2} yr^{-1})	489 ± 109	536 ± 110	444 ± 129	372 ± 13	0.309
LAI_{Aug} (m^2 m^{-2})	1.04 ± 0.41	1.07 ± 0.33	0.90 ± 0.34	1.16 ± 0.33	0.848
SLA_{Oct} (m^2 kg^{-1})	11.6 ± 0.7	11.4 ± 1.3	11.5 ± 0.6	9.1 ± 0.3	**0.020***
PNC (g g^{-1} × 100)	0.51 ± 0.03	0.49 ± 0.01	0.55 ± 0.05	0.68 ± 0.05	**0.003****
LNC_{Oct} (g g^{-1} × 100)	2.22 ± 0.12	2.11 ± 0.12	1.98 ± 0.08	2.03 ± 0.22	0.296
$LNLA_{Oct}$ (mmol m^{-2})	136 ± 4	133 ± 9	124 ± 11	158 ± 11	**0.013***
SNC_{Oct} (g g^{-1} × 100)	0.25 ± 0.01	0.24 ± 0.02	0.27 ± 0.02	0.31 ± 0.01	**0.001****
SNC_{Mar} (g g^{-1} × 100)	0.32 ± 0.02	0.34 ± 0.01	0.35 ± 0.03	0.41 ± 0.05	0.066
N’ (kg plot^{-1})	0.28 ± 0.07	0.23 ± 0.01	0.20 ± 0.03	0.26 ± 0.12	0.672
N_s (kg plot^{-1})	0.07 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.05 ± 0.01	0.502
U_N (kg kg^{-1})	4.01 ± 0.51	4.13 ± 1.13	4.75 ± 1.61	4.99 ± 0.97	0.672
E_N (kg kg^{-1} yr^{-1})	148 ± 24	169 ± 33	148 ± 31	108 ± 6	0.104
NAE (kg kg^{-1} yr^{-1})	1.88 ± 0.36	2.44 ± 1.09	2.65 ± 1.63	2.18 ± 0.44	0.811

** significant at the 0.01 level, * significant at the 0.05 level

[§] SB annual shoot biomass growth, R_{SB} relative shoot biomass growth, LAR leaf area ratio, LAP leaf area productivity, LAI leaf area index, SLA specific leaf area, PNC plant N concentration, LNC leaf N concentration, LNLA leaf N content per leaf area, SNC shoot N concentration, N’ mean plant (shoot + leaf) N content during the growing season, N_s shoot N pool prior to the start of the growing season, U_N N uptake efficiency, E_N shoot-specific N efficiency, NAE N accumulation efficiency; the subscripts Mar, Aug and Oct refer to the corresponding months of assessment variables. The effect of individual varieties on community response variables was evaluated by comparing the mean of all plots in which the respective variety was present, with the mean of the ones in which the same variety was absent; and including the plots with the corresponding monocultures and all mixtures with two and three varieties per plot. This comparison was performed using analysis of covariance (ANCOVA, GLM) with diversity level as a covariate. Slightly unbalanced data were treated by using adjusted sums of squares. Observed community-level trait values, based on the measured data from different diversity levels (2, 3 and 4 varieties) or the mixed plots in which a given variety was present, were also evaluated in relation to expected trait values, which were calculated by averaging the corresponding values for the individual varieties grown in pure culture and accounting for the realized share of each variety in the respective mixtures (i.e., expected community-scale traits are calculated as weighted averages of trait values for each diversity level or in each variety in the community). The statistical evaluation of the comparison between observed data and the corresponding expected values was made using one-sample T-tests (95% CI). All statistics were computed with SPSS software (Release 26, IBM SPSS, Chicago, IL).

3. Results

3.1. Biomass productivity and effects of diversity level and variety on functional traits

Annual shoot growth during the 2016 growing season varied between 16.9 kg plot^{-1} (or 1.8 Mg ha^{-1}) and 61.1 kg plot^{-1} (or 6.6 Mg ha^{-1}) across all 45 plots, with a mean of 36.7 kg plot^{-1} (or 4.0 Mg ha^{-1}) (Supplement S1). Considering only the plots with the pure cultures ($n = 3$), annual shoot growth and also relative shoot biomass annual increment (R_{SB}) were similar between the varieties; however, significant variation between the varieties was found with respect to some traits, mainly those at a lower level of organization and linked to biomass allocation and N use (Table 2).

Contrary to our first hypothesis, none of the functional traits listed in Table 2 (including shoot biomass growth and R_{SB}) was significantly affected by the diversity level (two-way ANOVA factors diversity level and block; $df = 3$ and $p \geq 0.297$ for diversity level). Table 3 shows that also almost all mean trait values observed for the three diversity levels (i.e., mixtures of 2, 3 and 4 varieties) were similar to the expected weighted average trait values from the pure cultures (Table 2), with weights calculated as the shares of each variety in the respective mixtures. The only exception was the leaf N concentration (LNC), which was significantly lower than the expected values in the mixtures of two and three varieties (Table 3). In addition, mixtures of the two varieties taxonomically distinct at the species level ('Tora' and 'Loden') achieved significantly higher N uptake efficiency (mean $U_N = 4.97$ kg N (kg N)$^{-1}$) compared to the corresponding mixture of the two full-siblings ('Tora' and 'Björn'; mean $U_N = 3.95$ kg N (kg N)$^{-1}$) (one-way ANOVA, $df = 2$, $p = 0.011$); but none of the other traits listed in Table 2 was significantly affected for this comparison.

3.2. Relationships between functional traits related to growth and nitrogen use

In this section, we assess correlations among traits, using eq. 1–5 to identify plausible correlations. Across all varieties and diversity levels, shoot biomass growth (SB) and relative shoot biomass increment (R_{SB}) were significantly correlated (Pearson $r = 0.457$, $p = 0.002$, $n = 45$). The R_{SB} was positively correlated with leaf area productivity (LAP), but not significantly correlated with leaf area ratio (LAR) (eq. 1; Table 4). The LAR was positively correlated to leaf biomass ratio (LBR; Pearson $r = 0.847$, $p < 0.001$, $n = 45$), but uncorrelated to specific leaf area (SLA) (eq. 2). The LAP increased with the shoot productivity per mean plant N (E_N), but was uncorrelated to the leaf N per leaf area (LNLA) (eq. 3). Further, the R_{SB} was strongly positively correlated to E_N, but not significantly correlated to the mean plant N concentration (PNC) (eq. 4) and the shoot N concentrations (SNC) in October and March. Higher N accumulation efficiency (NAE) was accomplished by increases in both N uptake efficiency (U_N) and E_N (eq. 5).

In addition to plausible correlations based on eq. 1–5, we also explored how traits were correlated to leaf area index (LAI) and the mean plant N pool (N'). The LAI in August was positively correlated to LAP (Pearson $r = 0.302$, $p = 0.044$, $n = 45$) and SB (Pearson $r = 0.655$, $p < 0.001$, $n = 45$); and a higher LAI was also associated with a higher stand-level plant N pool during the growing season (Pearson $r = 0.724$, $p < 0.001$, $n = 45$). While the shoot biomass growth increased steadily with N', albeit with diminishing returns at higher N', the LAP increased with N' up to a maximum and decreased thereafter (Fig. 1). In agreement with our second hypothesis, plots in which the variety 'Tora' was present achieved higher shoot biomass growth and LAP compared to the plots in which this variety was absent, but the highest values were not observed in the pure 'Tora' plots (Fig. 1). The relationship between E_N and N' showed the same pattern compared to the relationship between LAP and N', i.e., increasing E_N with N' up to a maximum at around 0.27 kg N plot^{-1} and decreasing E_N thereafter (quadratic regression $r^2 = 0.19$, $p = 0.014$, $n = 45$).

3.3. Effects of individual variety presence or absence on functional traits

As we hypothesized (*H2*), the presence or absence of individual varieties in a community (including the pure and mixed plots of up to three community components) had strong and significant effects on

Table 3

Means ± SE of expected and observed trait values assessed separately for each individual variety in plots of four different willow varieties grown in mixtures of three diversity levels (DL2, DL3, DL4) during the 2016 growing season in Central Sweden. Observed values are from measurements (means from all mixed plots with the same diversity level); and expected values are calculated as weighted averages of the values measured in the pure cultures (cf. Table 2), with weights equal to the realized share of each variety in the respective mixtures. The acronyms are explained in Table 2; SLA, LNC and SNC were measured in October 2016 while the other traits are complex traits integrating measurements at different sampling occasions between April 2016 and March 2017. Asterisks indicate significant (one-sample T-test, 95% CI) differences between the observed data and the corresponding expected figures (n.s. not significant, * $p \leq 0.050$, ** $p \leq 0.010$).

	Expected	Observed DL2 ($n = 18$ plots)	Observed DL3 ($n = 12$ plots)	Observed DL4 ($n = 3$ plots)
R_{SB} (yr^{-1})	3.29	3.16 ± 0.14 n.s.	3.33 ± 0.15 n.s.	3.17 ± 0.21 n.s.
LAR ($\text{m}^2\ \text{kg}^{-1}$)	2.12	2.03 ± 0.09 n.s.	2.09 ± 0.07 n.s.	2.06 ± 0.27 n.s.
LAP ($\text{g m}^{-2}\ \text{yr}^{-1}$)	460	477 ± 25 n.s.	474 ± 23 n.s.	476 ± 58 n.s.
SLA_{Oct} ($\text{m}^2\ \text{kg}^{-1}$)	10.9	10.7 ± 0.2 n.s.	10.9 ± 0.3 n.s.	10.6 ± 0.6 n.s.
PNC ($\text{g g}^{-1} \times 100$)	0.56	0.53 ± 0.02 n.s.	0.54 ± 0.01 n.s.	0.53 ± 0.03 n.s.
LNC_{Oct} ($\text{g g}^{-1} \times 100$)	2.09	2.01 ± 0.02 **	2.02 ± 0.03 *	1.92 ± 0.09 n.s.
SNC_{Oct} ($\text{g g}^{-1} \times 100$)	0.27	0.26 ± 0.01 n.s.	0.26 ± 0.01 n.s.	0.26 ± 0.01 n.s.
U_N (kg kg^{-1})	4.47	4.27 ± 0.18 n.s.	4.43 ± 0.19 n.s.	4.36 ± 0.31 n.s.
E_N ($\text{kg kg}^{-1}\ \text{yr}^{-1}$)	144	149 ± 7 n.s.	152 ± 6 n.s.	151 ± 10 n.s.
NAE ($\text{kg kg}^{-1}\ \text{yr}^{-1}$)	2.29	2.16 ± 0.14 n.s.	2.33 ± 0.15 n.s.	2.17 ± 0.21 n.s.

Table 4
Pearson correlation coefficients and significance levels for various growth and N use traits (explanation of acronyms see Table 2) assessed in plots of pure and mixed cultures of willow (4 varieties) grown in Central Sweden. The values in the upper right corner refer to the Pearson correlation coefficients (** significant at the 0.01 level, * significant at the 0.05 level), and the values in the lower left corner are the corresponding P values ($P \le 0.050$ in bold). n = 45.

	R_{SB}	LAR	LAP	SLA	LNLA	LNC	SNC_{Oct}	SNC_{Mar}	PNC	N_s	U_N	E_N	NAE
R_{SB}	.	0.015	0.567**	-0.179	0.163	0.008	0.013	0.199	0.122	-0.398**	0.842**	0.610**	0.999**
LAR	0.922	.	-0.780**	0.133	-0.039	0.093	0.599**	0.515**	0.815**	-0.502**	0.399**	-0.640**	0.015
LAP	**0.000**	**0.000**	.	-0.260	0.157	-0.098	-0.503**	-0.339*	-0.583**	0.170	0.196	0.908**	0.567**
SLA	0.240	0.383	0.085	.	-0.800**	-0.252	-0.329*	-0.334*	-0.324*	0.033	-0.241	0.073	-0.179
LNLA	0.285	0.801	0.304	**0.000**	.	0.371*	0.253	0.475**	0.476**	0.135	0.256	-0.192	0.163
LNC	0.956	0.545	0.522	0.095	**0.012**	.	-0.130	0.213	0.227	0.269	0.041	-0.156	0.008
SNC_{Oct}	0.933	**0.000**	**0.000**	**0.027**	0.094	0.394	.	0.622**	0.822**	-0.476**	0.326*	-0.645**	0.013
SNC_{Mar}	0.191	**0.000**	**0.023**	**0.025**	**0.001**	0.160	**0.000**	.	0.743**	-0.319*	0.226	-0.467**	0.199
PNC	0.423	**0.000**	**0.000**	**0.030**	**0.001**	0.134	**0.000**	**0.000**	.	-0.425**	0.501**	-0.683**	0.122
N_s	**0.007**	**0.000**	0.264	0.831	0.378	0.073	**0.001**	**0.033**	**0.004**	.	-0.508**	0.075	-0.398**
U_N	**0.000**	**0.007**	0.196	0.111	0.090	0.787	**0.029**	0.136	**0.000**	**0.000**	.	0.230	0.841**
E_N	**0.000**	**0.000**	**0.000**	0.636	0.206	0.306	**0.000**	**0.001**	**0.000**	0.626	0.128	.	0.610**
NAE	**0.000**	0.922	**0.000**	0.239	0.285	0.959	0.933	0.190	0.424	**0.007**	**0.000**	**0.000**	.

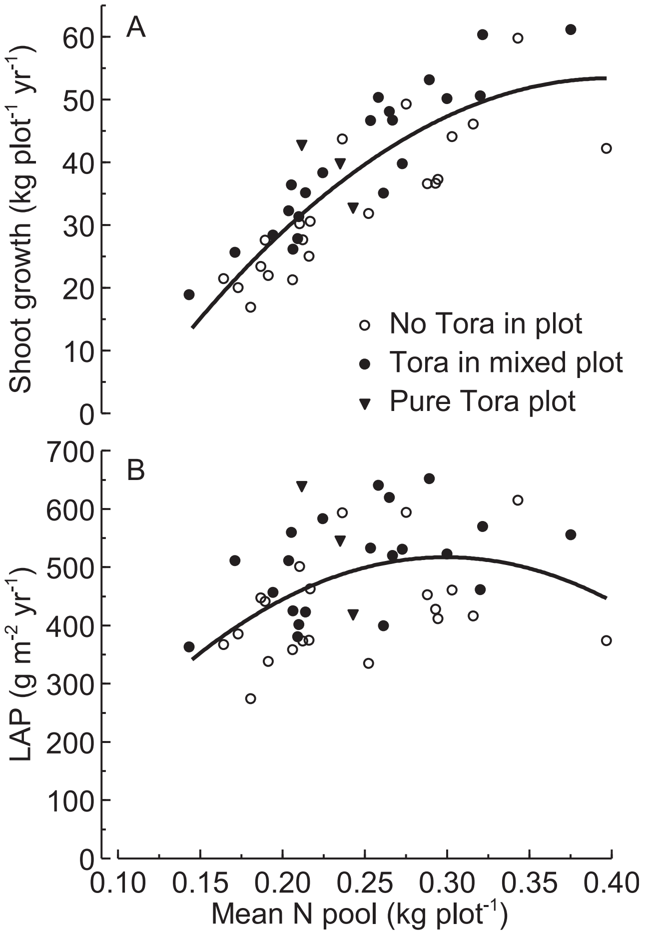


Fig. 1. Relationships between the stand-level mean plant nitrogen (N) pool and annual shoot biomass growth (A) or mean leaf area productivity (LAP) (B) for four willow varieties ('Björn', 'Tora', 'Jorr', 'Loden') grown in plots (9.6 m × 9.6 m, 144 trees per plot) of pure and mixed cultures during the 2016 growing season in Central Sweden. Different symbols indicate the cases in which 'Tora' was absent or present in a plot; the latter with further specification of pure 'Tora' plots. For further explanation see text. Regressions: (A) $y = 491.5 \times - 614.9 x^2 - 44.8$, $r^2 = 0.72$, $p < 0.001$, $n = 45$; (B) $y = 4431.8 \times - 7407.0 x^2 - 145.8$, $r^2 = 0.20$, $p = 0.010$, $n = 45$.

some functional traits both at low level of organization (e.g., SLA, LNC, PNC) and high level of organization (e.g., R_{SB}, LAP, U_N, E_N) (Fig. 2). For example, the presence of 'Tora' increased LAP, E_N and R_{SB}, but decreased mean PNC and SNC in October; whilst the presence of 'Loden' in a community increased the LAR, PNC, SNC and U_N, but decreased the SLA, LAP and E_N of the community. The presence of 'Jorr' in a community did not exert significant impacts on any of the traits except LNC; and the presence of 'Björn' decreased U_N and had partly contrasting effects on traits compared to the effects of its close relative (full-sib) 'Tora' (e.g., R_{SB} as well as NAE and its components). The significant differences were still apparent for all traits at high level of organization (but only few traits at low level) even for the case of including only the mixed plots of two or three community components and excluding the pure variety plots (Fig. 2). Most but not all of the mean trait values observed in the mixed plots in which a given variety was present (i.e., the closed bars in Fig. 2) were similar to the expected values calculated from the figures for the individual varieties grown in pure culture (Table 2) and the share of each variety in the respective mixtures (Fig. 3; calculations in Supplement S1). Exceptions were a significantly higher than expected E_N for 'Tora' (Fig. 3H), and lower than expected values of U_N for 'Björn' (Fig. 3G) and LNC for all varieties (Fig. 3E).

4. Discussion

4.1. Promising approach to explore functional trait relationships at stand level

In this paper we estimated the evolution of monthly means of stand-scale above ground biomass and nitrogen pools throughout one full growth cycle (12 months) to obtain growing-season means, which then were used for calculating some indices for nitrogen uptake and utilization efficiency reflecting patterns of nitrogen acquisition and conversion to shoot biomass. In addition, we related these indices to some traits at lower and higher levels of organization, and evaluated mechanistic links between them using elements of functional growth analysis; a methodology that is usually applied to small plants grown under (semi-)controlled conditions to not violate basic principles. The approach applied here combines the advantages of working at field scale, albeit only including above ground plant parts, with those of functional growth analysis (Hunt, 1982) providing an analytical framework to interpret the data. Although many of the functional relationships addressed here could not be interpreted in a strictly quantitative sense (i.e., eq. 1–4 are interpreted as proportionalities instead of equalities), we were able to utilize them for evaluating the relationships between traits that are known to be important for driving growth. Functional traits are considered important for explaining plant productivity (Lavorel and Garnier, 2002), and our approach appears promising for systematically analyzing the mechanistic links between growth and nitrogen use traits at community level in tree stands. One of the strengths of this study is therefore the choice of approach for linking traits to patterns of growth and nitrogen use at field scale. Another

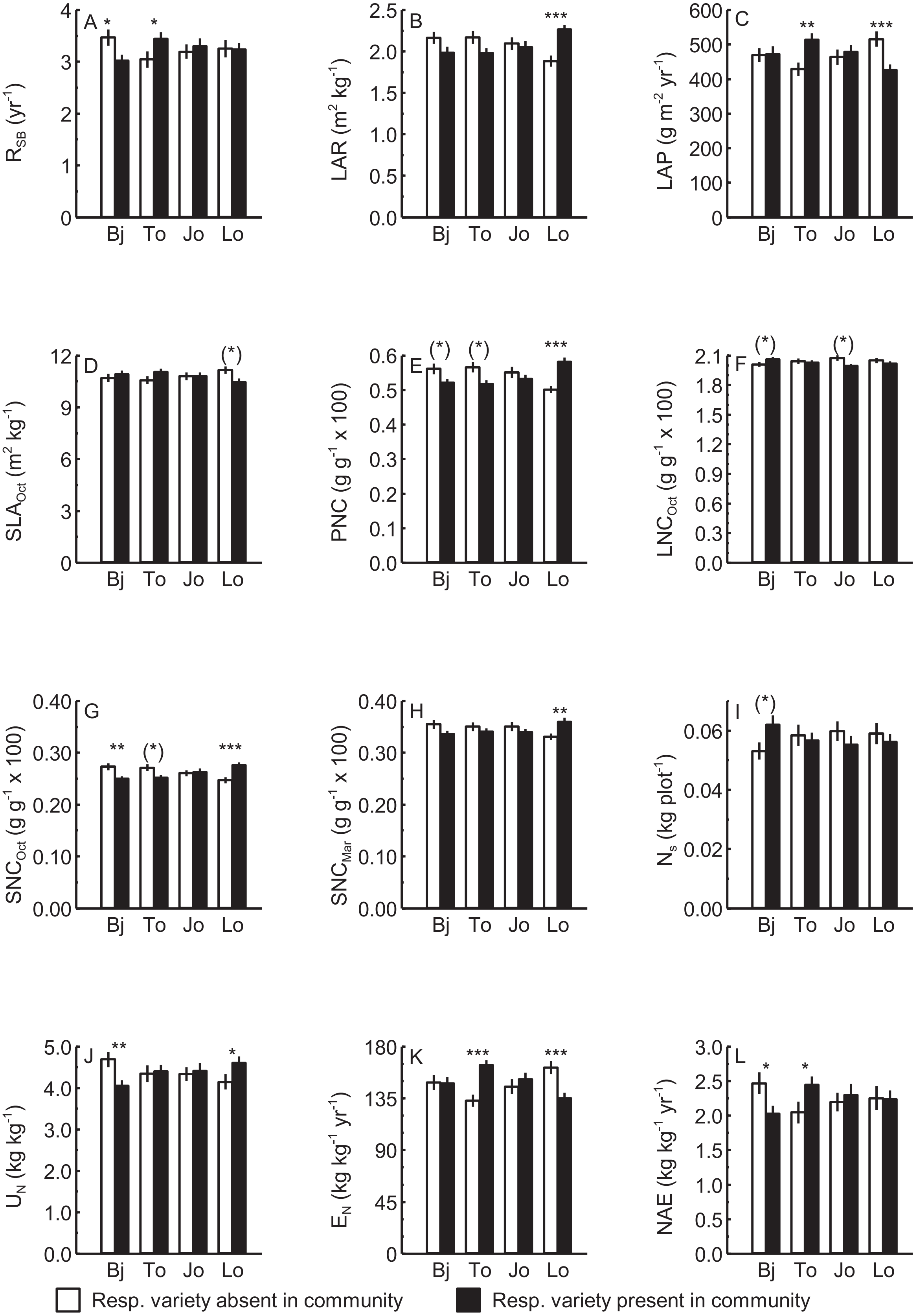


Fig. 2. Stand-level traits assessed separately for each individual variety in plots of four different willow varieties grown in pure and mixed culture of up to three varieties during the 2016 growing season in Central Sweden. Mean values are shown for the plots in which a given variety (Bj – 'Björn', To – 'Tora', Jo – 'Jorr', and Lo – 'Loden') was absent (open bars) and present (closed bars). The acronyms are explained in Table 2. Asterisks indicate significant (ANCOVA, covariate diversity level) differences between the means for the plots in which a given variety was absent or present (* $p \leq 0.050$, ** $p \leq 0.010$, *** $p \leq 0.001$). Asterisks in parentheses indicate those cases in which significant differences were observed only when the pure-culture plots were included in the analysis in addition to the mixed plots; but not when only the plots with the two- and three-mixtures were considered.

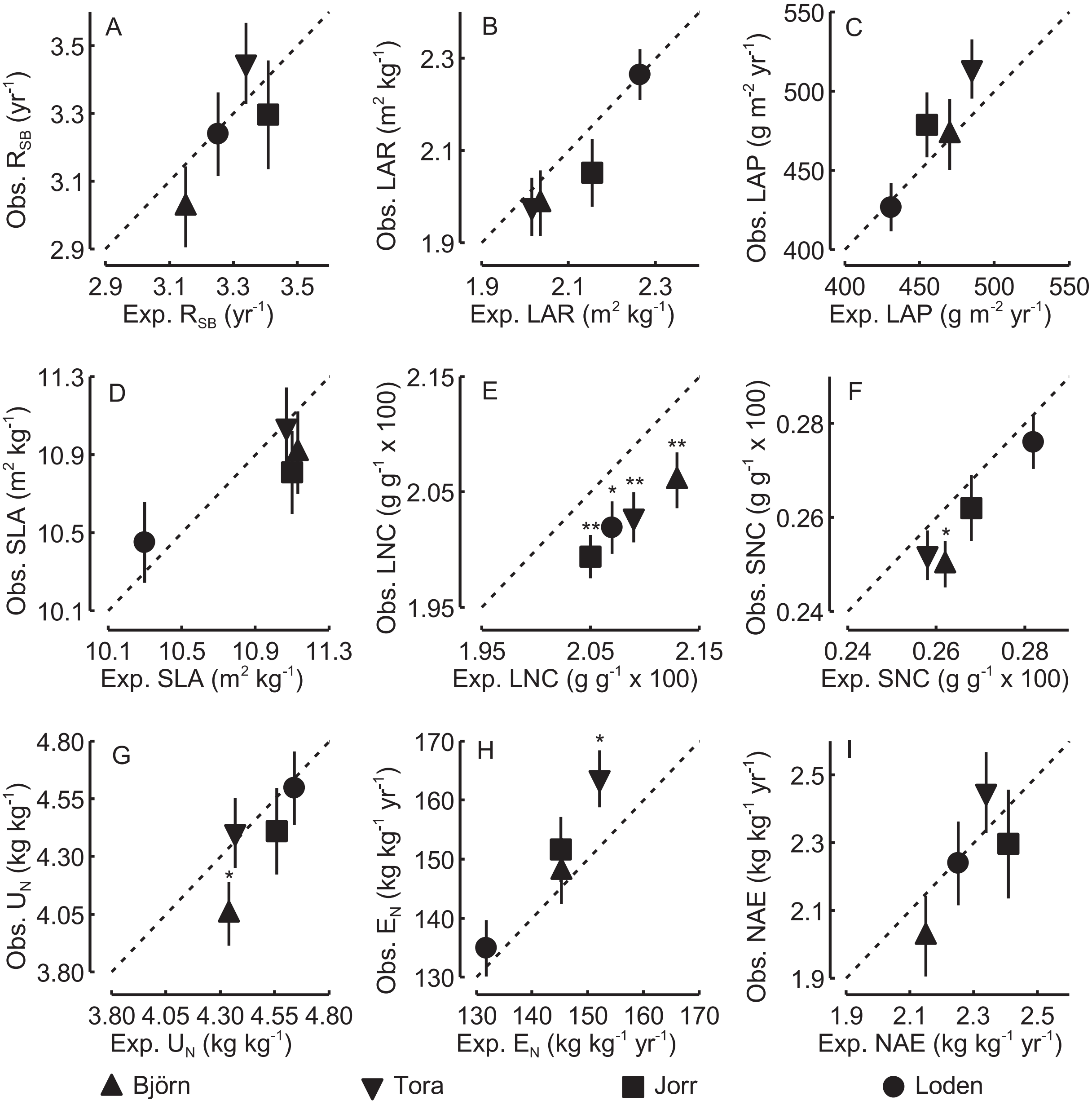


Fig. 3. Means ± SE of observed (Obs.) *vs.* expected (Exp.) trait values assessed separately for each individual variety in plots of four different willow varieties grown in pure and mixed culture of up to three varieties during the 2016 growing season in Central Sweden. Observed values are based on the measured data from the mixed plots in which a given variety ('Björn', 'Tora', 'Jorr', 'Loden') was present (i.e., the closed bars in Fig. 2); and expected values were calculated using the corresponding values for each variety measured when grown in pure culture (cf. Table 2) and the realized share of each variety in the respective mixtures. The broken lines indicate where observed values would equal expected values. The acronyms are explained in Table 2; SLA, LNC and SNC were measured in October 2016 while the other traits are complex traits integrating measurements at different sampling occasions between April 2016 and March 2017. Asterisks indicate significant (one-sample T-test, 95% CI, n = 21) differences between the observed data and the corresponding expected figures (* $p \leq 0.050$, ** $p \leq 0.010$).

advantage is the appropriate design of the field trial used here, offering a full-factorial diversity gradient including four contrasting varieties that are taxonomically distinct at species or genotype level. A limitation is the interpolation of biomass and nitrogen pools between the sampling occasions for which measured data were available (Supplement S1). However, a sensitivity analysis revealed that significance patterns with regard to the traits and indices used were not very sensitive to slight changes of the interpolation coefficients; i.e., significance patterns changed very little when slightly different coefficients for the monthly increases or decreases in biomass were chosen. We also cannot rule out that the temporal dynamics of biomass increases and decreases between the sampling points varied between the varieties, which would influence the result details but probably not the overall conclusions. Other limitations include the analysis of only one single location and year, although we know that, for example, the nitrogen uptake and use indices used here vary with the environmental conditions and between years in *Salix* (Weih et al., 2014, 2018). To recognize these limitations, here the main focus will be on the observed relationships between the trait profiles and nitrogen use patterns of the investigated varieties grown at various diversity levels; and the opportunities that these relationships offer for the management of short rotation willow plantations with regard to the sustainability aim of reduced use of synthetic nutrient fertilizers implying reduced energy use and carbon footprint (Higman et al., 2005; Hammar et al., 2017; Lutes et al., 2019).

4.2. No evidence for strong effects of diversity level on functional traits at stand-level

A lack of a strong diversity effect on the standing biomass in the willow trial investigated here was already documented previously (Hoeber et al., 2018), whilst the focus of the present study is beyond the previously observed patterns and on annual shoot growth increment in relation to the underlying trait relationships with respect to biomass allocation and nitrogen use. Plant functional traits and phenotypic plasticity have been suggested to modulate plant-plant interactions through influencing complementarity (Perez-Ramos et al., 2019), and we therefore expected to see significant effects of diversity level on some of the traits assessed here in pure and mixed willow stands. We also expected that the diversity effect is more prominent for traits assessed at high than low level of organization, due to accumulated plasticity (from multiple underlying traits) and thereby greater complementarity for the traits at higher organization level. However, none of the traits investigated varied significantly between the diversity levels, in spite of some significant differences for those traits between the varieties both when they were grown in pure plots (Table 2) and when they were admixed (Fig. 2). In theory, it is possible that the plastic responses of individual varieties to different neighbors cancelled out each other due to spatial averaging effects. This explanation cannot be ruled out because we assessed many traits at an aggregated community level as also others have done (Garnier et al., 2004). It appears however unlikely that the possible plastic responses cancelled out in the vast majority of all mixtures, although the presence (or absence) of some of the varieties had clear and significant effects on the same traits (e.g., LAR and LAP) when these varieties were grown in mixture.

The field trial investigated here was unfertilized, which is also reflected by the relatively low yield figures (Hoeber et al., 2018). Low-resource environments have been suggested to favor complementary resource use by providing a wider range of niches, and many studies have shown so-called overyielding (i.e., higher yields than would be expected from the corresponding figures achieved by the same mixture components grown in pure cultures) to more often occur on poor sites than rich sites (Tilman et al., 1997; Tilman, 1999). We found no significant diversity effect on any of the growth traits, nitrogen uptake and use indices investigated in this study, and therefore no clear evidence in support of our first hypothesis (*H1*).

4.3. Admixture of some varieties strongly affects community productivity and functional traits

In contrast to the investigation by Hoeber et al. (2018), which encompassed the entire first cutting cycle, the present investigation was restricted to the final year of the first cutting cycle, which was the first year in which full canopy closure was achieved and resource competition by neighboring plants has probably occurred. The ANOVA-like Bayesian approach applied by Hoeber et al. (2018) is based on probability estimates and distributions, which seem more appropriate for the longer-term perspective of a whole cutting cycle but less appropriate for the annual perspective of the present investigation. Both studies provide convincing evidence that the characteristics of individual varieties strongly affect stand-level growth and productivity, but some of the specific patterns differ between the whole-cutting cycle perspective by Hoeber et al. (2018) and the one-year perspective explored here with a different methodology. For example, when analyzed with the ANOVA-like Bayesian approach by Hoeber et al. (2018), the variety 'Tora' was predicted to reduce community biomass production in mixed plots while its own productivity was predicted to benefit from mixing. In contrast, admixing 'Tora' resulted in significantly increased annual shoot biomass increment in the present analysis with focus on only the last year of the cutting cycle (Fig. 2). The results suggest that the relative advantage of 'Tora' compared to the other varieties, reflected by its benefit from mixing, started in the final year of the cutting cycle to over-compensate its initially negative effect on stand productivity. Such a pattern would be typical for a 'selection effect' which has been described as a community-based mechanism for improving productivity in mixed communities (Loreau and Hector, 2001); as opposed to the 'complementarity effect' due to niche differentiation which is the main interest of the present investigation.

In this field trial, nutrient (e.g. nitrogen) availability or acquisition is considered the main growth-limiting factor, and any variation in light use efficiency through, e.g., differences in canopy architecture or leaf phenology would imply variation in the efficiency of conversion of the most limiting resource to shoot biomass. In addition, several studies identified the competition for light as size-asymmetric (Schwinning and Weiner, 1998; del Rio et al., 2014), implying that varieties with larger individuals would take more than their (size-) proportional share of intercepted light compared to smaller varieties; and that possible size advantages achieved through greater conversion efficiency are likely to increase over time. In this context, the variety 'Tora' is again an interesting case, because it was shown to enhance stand-level productivity in both pure plots and those plots in which it was admixed, by achieving greater productivity per leaf area (LAP; eq. 1) and plant nitrogen (E_N; eq. 4) (Figs. 2, 3H). This pattern also confirms the similar relationships identified for this variety in a controlled pot study (Weih, 2001). In another study characterizing various willow varieties in terms of morphology and physiology, Robinson et al. (2004) noted the openness of the canopy of 'Tora', which has also a weak vertical leaf nitrogen gradient and a deep foliated canopy (Weih and Ronnberg-Wastljung, 2007). Thus, the uppermost leaves of 'Tora' often hang down almost vertically, whilst the leaves at lower-canopy levels are close to horizontally oriented – an architecture that is likely to support high conversion efficiency *sensu* LAP and E_N. Thus, we show here, for an early-successional tree, evidence for admixing effects through the increase of conversion efficiency (i.e., 'Tora'), whilst intercrops in agriculture have been concluded to mostly improve acquisition rather than conversion efficiency (Stomph et al., 2020).

Our results showed that the pure plots, with only one variety, normally were not superior in terms of, e.g., shoot biomass and productivity per unit nitrogen (exemplified by 'Tora' in Fig. 1). Further, the admixture of different varieties modified various traits, especially those at a high level of organization (e.g., LAP, E_N) as opposed to the traits at low level (e.g., SLA, PNC, LNC) (Fig. 2). In addition, most but not all of the admixing effects on traits could be explained by the effects of the individual variety characteristics combined with their relative share in the respective mixtures (Fig. 3). Exceptions from this pattern were the positive admixing effect of 'Tora' on nitrogen conversion efficiency (E_N), the negative admixing effect of 'Björn' on nitrogen acquisition efficiency (U_N), and the negative admixing effects on leaf nitrogen concentrations seen for all varieties. Those cases could represent examples for trait plasticity modulating plant-plant interactions and contributing to increased complementarity (Perez-Ramos et al., 2019). Evidence for plastic changes of growth traits in response to neighbors of different genotype identities has been documented previously for some *Salix* spp. and interpreted as adaptations to local environmental conditions (Grady et al., 2017). Also the plastic changes of nitrogen economy traits, among them leaf nitrogen concentration, in response to admixing different cultivars of barley (Dahlin et al., 2020) indicate that plastic responses modulating plant-plant interactions are not unusual and could have contributed to the mixture performances in our study. Therefore, our results support our second hypothesis (*H2*) that the presence or absence of individual varieties in a mixture significantly affects the growth and nitrogen use traits observed in that mixture. Moreover, the mean values of some traits observed in a mixture differ from the corresponding values expected from assessing all individual community components grown in pure cultures.

4.4. Teaming up willow varieties for sustainable biomass production in low-input conditions

In young willow plantations, the diversity level *per se* appears not to have significant effects on the traits related to growth and nitrogen economy, with respect to both the entire first cutting cycle (Hoeber et al., 2018) or only its last year as is shown in this study. However, the identity of the component admixed to a stand is critical, because admixing of some trait profiles (e.g., varieties) related to, for example, conversion efficiency seem to favor stand productivity more than others. These results are in line with the observation that, in managed ecosystems, greater diversity is not necessarily associated with higher productivity especially when compared to the best pure culture (Schulze et al., 2018). In commercial willow short rotation systems, management probably cannot rely on achieving high productivity through simply adding more varieties, but needs to carefully consider the trait profiles of the admixed components to support sustainability goals related to productivity, resource use efficiency and other ecological processes linked to ecosystem services. In regard to productivity and resource use efficiency, there is no doubt that nutrient fertilization increases willow productivity in many sites, albeit depending on the plant material and site conditions (Weih and Nordh, 2005; Fabio and Smart, 2018b). However, it has been shown for willow and many other plants that nutrient fertilization usually decreases the productivity per unit of nutrient resource (Tilman et al., 2002; Weih et al., 2018), thus decreasing resource use efficiency. For these reasons, the non-fertilized conditions used in our study comply well with the sustainability goals (Holden et al., 2014) and also the recommendations to establish willow short rotation coppice in low-input conditions to avoid competition with food and feed production land (Knur et al., 2008). In these circumstances, mixed willow stands, either in row-based (Dillen et al., 2016) or individual-based mixing designs (Hoeber et al., 2018) appear desirable alternatives to the most frequently applied pure stands, as they most likely confer no disadvantage in terms of productivity and, at the same time, offer additional opportunities for ecosystem services related to, e.g., cropping security and pest or disease resistance (McCracken and Dawson, 1994, 1998; Grossman et al., 2018).

In this study, we found admixing effects through either the increase of conversion efficiency (e.g., 'Tora') or the enhancement of acquisition efficiency (e.g., 'Loden'). To benefit ecological sustainability, increasing the conversion efficiency (e.g., LAP or E_N) of a mixed stand through admixture of an appropriate variety appears most meaningful, because the alternative of increasing acquisition efficiency (e.g., U_N) would, in many cases, be associated with greater demand for synthetic nutrient fertilizers. Based on our results, we suggest designing teams of varieties that, on the one hand, enhance stand-level E_N and LAP (e.g., admixing varieties with a trait profile similar to 'Tora'), and, on the other hand, could confer additional benefits not addressed here such as the enhancement of soil carbon sequestration (Gregory et al., 2018; Baum et al., 2020). The trait values of individual varieties assessed in monocultures could be used to guide the design of mixtures, although our results (e.g., Fig. 3) indicate that also additional effects of plant-plant interactions between different varieties can modify the trait expressions of individual varieties grown in mixtures. However, more investigations on different soil and climatic conditions, integrating multiple years and cutting cycles and using other plant material, need to be performed to further explore and verify the observations made in this investigation and convert the knowledge into practical guidelines for management.

5. Conclusions

Functional traits are considered important for explaining plant productivity, and our approach of linking traits to patterns of growth and nitrogen use at stand level appears promising for systematically analyzing the mechanistic links between growth and nitrogen use traits at community level in tree stands. Diversity level had no significant effect on the traits assessed here, and we thus found no evidence in support of our first hypothesis that traits linked to growth, nitrogen uptake and use are significantly affected by the diversity level *per se*. The absence or presence of individual varieties strongly affected various trait values, and the admixing effects on traits were in most (but not all) cases explained by the trait values of the individual varieties assessed in monocultures in combination with their relative share in the respective mixtures. Desirable variety mixtures could be designed that combine, for example, the high conversion efficiency that certain varieties achieve in mixed stands with the specific nutrient acquisition characteristics of other varieties.

CRediT authorship contribution statement

Martin Weih: Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Writing - original draft. **Nils-Erik Nordh:** . **Stefano Manzoni:** Conceptualization, Writing - review & editing. **Stefanie Hoeber:** Data curation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The establishment and management of the field trial was funded by grants from the Swedish Energy Agency (projects no. 36654-1 and 36654-2). This research was partly funded by grants from the Swedish research council Formas (grant number 2016-00998) and the European Union's Horizon 2020 research and innovation programme under grant agreement No. 727284 (DIVERSify).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2020.118605.

References

Baum, C., Amm, T., Kahle, P., Weih, M., 2020. Fertilization effects on soil ecology strongly depend on the genotype in a willow (Salix spp.) plantation. For. Ecol. Manage. 466, 118126.

Dahlin, I., Kiaer, L.P., Bergkvist, G., Weih, M., Ninkovic, V., 2020. Plasticity of barley in response to plant neighbors in cultivar mixtures. Plant Soil 447, 537–551.

del Rio, M., Condes, S., Pretzsch, H., 2014. Analyzing size-symmetric vs. size-asymmetric and intra- vs. inter-specific competition in beech (Fagus sylvatica L.) mixed stands. For. Ecol. Manage. 325, 90–98.

Dickmann, D.I., Kuzovkina, J., 2014. Poplars and Willows of the World, With Emphasis on Silviculturally Important Species. In: Isebrands, J.G., Richardson, J. (Eds.), Poplars and Willows: Trees for Society and the Environment. Cabi Publishing, Wallingford, pp. 8–91.

Dillen, M., Vanhellemont, M., Verdonckt, P., Maes, W.H., Steppe, K., Verheyen, K., 2016. Productivity, stand dynamics and the selection effect in a mixed willow clone short rotation coppice plantation. Biomass Bioenergy 87, 46–54.

Fabio, E.S., Smart, L.B., 2018a. Differential growth response to fertilization of ten elite shrub willow (Salix spp.) bioenergy cultivars. Trees-Structure and Function 32, 1061–1072.

Fabio, E.S., Smart, L.B., 2018b. Effects of nitrogen fertilization in shrub willow short rotation coppice production - a quantitative review. Global Change Biology Bioenergy 10, 548–564.

Garnier, E., Cortez, J., Billes, G., Navas, M.L., Roumet, C., Debussche, M., Laurent, G., Blanchard, A., Aubry, D., Bellmann, A., Neill, C., Toussaint, J.P., 2004. Plant functional markers capture ecosystem properties during secondary succession. Ecology 85, 2630–2637.

Grady, K.C., Wood, T.E., Kolb, T.E., Hersch-Green, E., Shuster, S.M., Gehring, C.A., Hart, S.C., Allan, G.J., Whitham, T.G., 2017. Local biotic adaptation of trees and shrubs to plant neighbors. Oikos 126, 583–593.

Gregory, A.S., Dungait, J.A.J., Shield, I.F., Macalpine, W.J., Cunniff, J., Durenkamp, M., White, R.P., Joynes, A., Richter, G.M., 2018. Species and Genotype Effects of Bioenergy Crops on Root Production, Carbon and Nitrogen in Temperate Agricultural Soil. Bioenergy Res. 11, 382–397.

Grossman, J.J., Vanhellemont, M., Barsoum, N., Bauhus, J., Bruelheide, H., Castagneyrol, B., Cavender-Bares, J., Eisenhauer, N., Ferlian, O., Gravel, D., Hector, A., Jactel, H., Kreft, H., Mereu, S., Messier, C., Muys, B., Nock, C., Paquette, A., Parker, J., Perring, M.P., Ponette, Q., Reich, P.B., Schuldt, A., Staab, M., Weih, M., Zemp, D.C., Scherer-Lorenzen, M., Verheyen, K., 2018. Synthesis and future research directions linking tree diversity to growth, survival, and damage in a global network of tree diversity experiments. Environ. Exp. Bot. 152, 68–89.

Hammar, T., Hansson, P.A., Sundberg, C., 2017. Climate impact assessment of willow energy from a landscape perspective: a Swedish case study. Global Change Biology Bioenergy 9, 973–985.

Higman, S., Mayers, J., Bass, S., Judd, N., Nussbaum, R., 2005. The sustainable forestry handbook. Earthscan, London, UK.

Hoeber, S., Arranz, C., Nordh, N.E., Baum, C., Low, M., Nock, C., Scherer-Lorenzen, M., Weih, M., 2018. Genotype identity has a more important influence than genotype diversity on shoot biomass productivity in willow short-rotation coppices. Global Change Biology Bioenergy 10, 534–547.

Hoeber, S., Fransson, P., Prieto-Ruiz, I., Manzoni, S., Weih, M., 2017. Two Salix Genotypes Differ in Productivity and Nitrogen Economy When Grown in Monoculture and Mixture. Front. Plant Sci. 8, 12.

Holden, E., Linnerud, K., Banister, D., 2014. Sustainable development: Our Common Future revisited. Global Environmental Change-Human and Policy Dimensions 26, 130–139.

Holzwarth, F., Ruger, N., Wirth, C., 2015. Taking a closer look: disentangling effects of functional diversity on ecosystem functions with a trait-based model across hierarchy and time. R. Soc. Open Sci. 2, 19.

Hunt, R., 1982. Plant growth curves. The functional approach to plant growth analysis.

Ingestad, T., Agren, G.I., 1995. Plant nutrition and growth: Basic principles. Plant Soil 169, 15–20.

Karp, A., Shield, I., 2008. Bioenergy from plants and the sustainable yield challenge. New Phytol. 179, 15–32.

Knur, L., Murn, Y., Murach, D., 2008. Potenziale zur energetischen Nutzung von Agrarholz. In: Murach, D., Knur, L., Schultze, M. (Eds.), DENDROM Zukunftsrohstoff Dendromasse – Final report. Verlag Kessel, Remagen-Oberwinter, Germany, pp. 398–414.

Lambers, H., Freijsen, N., Poorter, H., Hirose, T., Vanderwerf, A., 1990. Analyses of growth based on net assimilation rate and nitrogen productivity - Their physiological background. In: Lambers, H. (Ed.), Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants. SPB Academic, The Hague, pp. 1–17.

Lavorel, S., Garnier, E., 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Funct. Ecol. 16, 545–556.

Loreau, M., Hector, A., 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412, 72–76.

Lutes, K., Oelbermann, M., Thevathasan, N.V., Gordon, A.M., 2019. Assessing the impact of fertilizer application on net soil-derived emission budgets from a temperate willow (Salix miyabeana) short rotation coppice system. Biomass Bioenergy 120, 135–143.

McCracken, A.R., Dawson, W.M., 1994. Experiences in the use of mixed-clonal stands of Salix as a method of reducing the impact of rust diseases. Norwegian Journal of Agricultural Sciences (Suppl.) 0, 101–109.

McCracken, A.R., Dawson, W.M., 1998. Short rotation coppice willow in Northern Ireland since 1973: Development of the use of mixtures in the control of foliar rust (Melampsora spp.). Eur. J. For. Pathol. 28, 241–250.

Perez-Ramos, I.M., Matias, L., Gomez-Aparicio, L., Godoy, O., 2019. Functional traits and phenotypic plasticity modulate species coexistence across contrasting climatic conditions. Nature. Communications 10.

Ra, K., Shiotsu, F., Abe, J., Morita, S., 2012. Biomass yield and nitrogen use efficiency of cellulosic energy crops for ethanol production. Biomass Bioenergy 37, 330–334.

Robinson, K.M., Karp, A., Taylor, G., 2004. Defining leaf traits linked to yield in short-rotation coppice Salix. Biomass Bioenergy 26, 417–431.

Schulze, E.D., Bouriaud, O., Weber, U., Roscher, C., Hessenmoeller, D., Kroiher, F., Schall, P., 2018. Management breaks the natural productivity-biodiversity relationship in forests and grassland: an opinion. Forest. Ecosystems 5.

Schwinning, S., Weiner, J., 1998. Mechanisms determining the degree of size asymmetry in competition among plants. Oecologia 113, 447–455.

Shipley, B., Vile, D., Garnier, E., 2006. From plant traits to plant communities: A statistical mechanistic approach to biodiversity. Science 314, 812–814.

Siefert, A., Violle, C., Chalmandrier, L., Albert, C.H., Taudiere, A., Fajardo, A., Aarssen, L.W., Baraloto, C., Carlucci, M.B., Cianciaruso, M.V., Dantas, V.D.L., de Bello, F., Duarte, L.D.S., Fonseca, C.R., Freschet, G.T., Gaucherand, S., Gross, N., Hikosaka, K., Jackson, B., Jung, V., Kamiyama, C., Katabuchi, M., Kembel, S.W., Kichenin, E., Kraft, N.J.B., Lagerstrom, A., Le Bagousse-Pinguet, Y., Li, Y., Mason, N., Messier, J., Nakashizuka, T., McC Overton, J., Peltzer, D.A., Perez-Ramos, I.M., Pillar, V.D., Prentice, H.C., Richardson, S., Sasaki, T., Schamp, B.S., Schoeb, C., Shipley, B., Sundqvist, M., Sykes, M.T., Vandewalle, M., Wardle, D.A., 2015. A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. Ecol. Lett. 18, 1406–1419.

Stomph, T., Dordas, C., Baranger, A., Rijk, J.d., Dong, B., Evers, J., Gu, C., Li, L., Simon, J., Jensen, E.S., Wang, Q., Wang, Y., Wang, Z., Xu, H., Zhang, C., Zhang, L., Zhang, W., Bedoussac, L., Werf, W.v.d., 2020. Designing intercrops for high yield, yield stability and efficient use of resources: are there principles? Adv. Agron. 160, 1–50.

Tilman, D., 1999. The ecological consequences of changes in biodiversity: A search for general principles. Ecology 80, 1455–1474.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature 418, 671–677.

Tilman, D., Lehman, C.L., Thomson, K.T., 1997. Plant diversity and ecosystem productivity: Theoretical considerations. PNAS 94, 1857–1861.

Turnbull, L.A., Levine, J.M., Loreau, M., Hector, A., 2013. Coexistence, niches and biodiversity effects on ecosystem functioning. Ecol. Lett. 16, 116–127.

Weih, M., 2001. Evidence for increased sensitivity to nutrient and water stress in a fast-growing hybrid willow compared with a natural willow clone. Tree Physiol. 21, 1141–1148.

Weih, M., Asplund, L., Bergkvist, G., 2011a. Assessment of nutrient use in annual and perennial crops: A functional concept for analyzing nitrogen use efficiency. Plant Soil 339, 513–520.

Weih, M., Bonosi, L., Ghelardini, L., Ronnberg-Wastljung, A.C., 2011b. Optimizing nitrogen economy under drought: increased leaf nitrogen is an acclimation to water stress in willow (Salix spp.). Ann. Bot. 108, 1347–1353.

Weih, M., Glynn, C., Baum, C., 2019. Willow Short-Rotation Coppice as Model System for Exploring Ecological Theory on Biodiversity-Ecosystem Function. Diversity-Basel 11.

Weih, M., Hamner, K., Pourazari, F., 2018. Analyzing plant nutrient uptake and utilization efficiencies: comparison between crops and approaches. Plant Soil 430, 7–21.

Weih, M., Hansson, P.A., Ohlsson, J.A., Sandgren, M., Schnurer, A., Ronnberg-Wastljung, A.C., 2020. Sustainable production of willow for biofuel use. In: Saffron, C. (Ed.), Achieving carbon-negative bioenergy systems from plant materials. Burleigh Dodds Science Publishing, Cambridge, UK, pp. 305–339.

Weih, M., Hoeber, S., Beyer, F., Fransson, P., 2014. Traits to ecosystems: the ecological sustainability challenge when developing future energy crops. Front. Energy Res. 2, 1–5.

Weih, M., Nordh, N.E., 2002. Characterising willows for biomass and phytoremediation: growth, nitrogen and water use of 14 willow clones under different irrigation and fertilisation regimes. Biomass Bioenergy 23, 397–413.

Weih, M., Nordh, N.E., 2005. Determinants of biomass production in hybrid willows and prediction of field performance from pot studies. Tree Physiol. 25, 1197–1206.

Weih, M., Ronnberg-Wastljung, A.C., 2007. Shoot biomass growth is related to the vertical leaf nitrogen gradient in Salix canopies. Tree Physiol. 27, 1551–1559.

Verheyen, K., Vanhellemont, M., Auge, H., Baeten, L., Baraloto, C., Barsoum, N., Bilodeau-Gauthier, S., Bruelheide, H., Castagneyrol, B., Godbold, D., Haase, J., Hector, A., Jactel, H., Koricheva, J., Loreau, M., Mereu, S., Messier, C., Muys, B., Nolet, P., Paquette, A., Parker, J., Perring, M., Ponette, Q., Potvin, C., Reich, P., Smith, A., Weih, M., Scherer-Lorenzen, M., 2016. Contributions of a global network of tree diversity experiments to sustainable forest plantations. Ambio 45, 29–41.

Verwijst, T., Telenius, B., 1999. Biomass estimation procedures in short rotation forestry. For. Ecol. Manage. 121, 137–146.