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Interactions of nutrient and water availability control growth and diversity effects in a *Salix* two-species mixture

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Abstract

Plant growth is constrained by resource availability and interactions among limiting resources—abundance in one resource (e.g., nutrients) might promote growth, thereby causing the depletion of other resources (e.g., water), potentially inducing stress or mortality. In a diverse plant community, complementary resource use has been hypothesized to increase the overall productivity, but how diversity effects vary with interacting water and nutrient limitation and through time is not known. Here, we address this knowledge gap in a controlled pot experiment where species composition (two *Salix* species in monoculture or mixture), nutrient addition, and watering frequency (for fixed total water inputs) were varied during two growing seasons. High nutrient availability promoted plant growth and nitrogen accumulation at the pot scale, as well as increased allocation aboveground, but also triggered more intense water stress and mortality, as larger plants depleted soil water during warm periods. Supplying water more frequently slightly alleviated water stress under high nutrient availability, thus promoting growth and nitrogen accumulation. The species mixtures performed better than the average of the mixture constituents (positive net diversity effects) and increasingly so through time. The complementarity and selection effects, respectively, increased and decreased under both high nutrient availability and high watering frequency. Overall, these results suggest that as plants grow larger, plant interactions and resource partitioning intensify, causing the positive diversity effects, but also that drought consequences might be exacerbated in plant communities rapidly growing thanks to high nutrient supply.

KEYWORDS

complementarity effects, drought mortality, net diversity effects, nutrient limitation, plant allocation, rainfall frequency, selection effects, tree diversity, water stress

1 | INTRODUCTION

Water and nutrients are major limiting factors for plant productivity. With the ongoing drought lengthening and intensification of rainfall events (Breinl et al., 2020; IPCC, 2021), water scarcity is becoming an ever more important cause of reduced productivity and increased

mortality (Allen et al., 2010). At the same time, soil nutrient availability is changing due to nutrient depletion in intensely managed land and increased nutrient deposition in some areas (Bouwman et al., 2009). These ongoing resource availability changes are posing challenges to global vegetation in both natural and managed ecosystems.

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Plants allocate growth to acquire the most limiting resource, trying to equalize the marginal return over the marginal cost of all its organs, so that “growth is equally limited by all resources” (Bloom et al., 1985, page 367). These plastic responses lead to higher allocation of biomass belowground when nutrients (e.g., Tilman & Wedin, 1991) and water (Chaves et al., 2003) are limiting. These allocation patterns are often associated with smaller leaf area (and higher leaf thickness) and altered leaf nutrient content. Although leaf nutrient content declines under nutrient limitation, it often increases under dry conditions, as plants acclimate to better exploit the available nutrient resources when leaf area and stomatal conductance are reduced (Wright et al., 2003). High nutrient availability is, in this case, beneficial to ameliorate drought responses, as it allows maintenance of photosynthetic capacity even when stomatal conductance is low. However, nutrients and water can also interact in a different way—in the presence of abundant nutrients, plants would grow faster, thus requiring more of other resources such as water. It is thus possible that relieving plants from nutrient limitation actually worsens the effect of drought.

Although these plant level responses are relatively well-known, the responses at the plant community level are less understood because of interspecific interactions. Theories for comparing the yield of monocultures and mixtures have been proposed since the 1960s (de Wit, 1960; van den Bergh, 1968). Early studies already recognized that the yield of the mixtures can be higher than expected based on the yield of the monocultures (Fowler, 1986) because species mixtures can express a wider range of functional traits compared with a single species (which is limited by its allocation plasticity) and thus use resources more effectively than monocultures. This suggests that the resource use of different species may be complementary, resulting in improved biogeochemical cycling and productivity at high diversity (Isbell et al., 2011; Loreau, 2000; Loreau & Hector, 2001) or lower rates when diversity is reduced (Naeem et al., 1994). Although complementarity effects (i.e., changes in community production compared with the average monoculture due to species interactions) have been widely documented, the mechanisms promoting complementarity are still debated (Loreau, 2000; Tilman et al., 1997; Turnbull et al., 2012; Yachi & Loreau, 2007). Niche segregation is one such mechanism—different species access resources in spatially separated environments or at different times, thus avoiding direct competition for those resources (in the case of water as limiting resource, see Silvertown et al., 2015). Other proposed mechanisms promoting coexistence and complementarity are different response speeds to disturbances (as shown in seagrass communities; Hughes & Stachowicz, 2011), reduction in competition strength (Loreau & de Mazancourt, 2013), and ameliorating effects of a mixed community on the environment (Wright et al., 2021).

The feedbacks between plant diversity and resource availability have been studied for the limiting resources we consider here—water and soil nutrients—but these resources have been mainly considered in isolation. Fast-growing species show strong growth responses to enhanced nutrient availability, possibly resulting in a low-diversity

community; in contrast, low resource environments may favour diversity by providing a wider range of niches (Tilman et al., 1996) and promoting niches defined by a high number of resources, which are best exploited by a diverse community (Harpole et al., 2016). In turn, a diverse community is able to stabilize soil nutrient availability and reduce leaching (Tilman et al., 1997). Higher variability in soil water also promotes diversity (Knapp et al., 2002), and more diverse communities can intensify the overall water use by accessing different soil water compartments (Krämer & Hölscher, 2010; Leimer et al., 2014; Silvertown et al., 2015) and promote survival of water stress sensitive species by ameliorating environmental conditions during dry periods (Wright et al., 2021). Therefore, it seems that low or variable resource availability would allow stronger complementarity effects to emerge.

Using plant mixtures could thus be useful to design sustainable agroecosystems that require less fertilizers and water (Malezieux et al., 2009; Verheyen et al., 2016) and to ‘protect’ species that are sensitive to water stress (Wright et al., 2021). Notably, it is often the occurrence of specific traits in the community that drives the community-scale performance (Gebauer et al., 2012; Hoerber et al., 2018; Tatsumi, 2020; Weih et al., 2021) and biogeochemical cycling (Hoerber et al., 2020), suggesting that species identity could be a more important determinant of diversity effects than species richness per se. Although diversity effects at various levels of individual resource availability have been studied (Craven et al., 2016), less is known on how interactions of nutrient and water availability might shape community responses to resource limitation.

The effects of plant interactions become increasingly important with time, as interactions intensify with larger plants that occupy the available niches (Cardinale et al., 2007). Complementarity effects accordingly increase through time, whereas selection effects (i.e., changes in community production compared with the average monoculture due to individual species) tend to be less sensitive (Cardinale et al., 2007) or become more negative (Tatsumi, 2020). However, little is known about the interactions between resource limitation and time on these diversity effects—is complementarity increasing more or less through time under contrasting nutrient and water availabilities?

Based on this evidence, we hypothesize that

HP1. Regardless of diversity level, lower nutrient and more intermittent water availability increase allocation to belowground biomass and result in thicker, smaller leaves—in turn, these changes reduce productivity but allow preserving soil water and prevent water stress (i.e., interactive effects between nutrient and water availability).

HP2. Higher diversity promotes more efficient resource acquisition, as reflected by increased complementarity effects, in particular under low nutrient availability and more variable soil water (longer dry periods followed by intense rewetting).

HP3. Complementarity effects increase through time as the plants grow larger due to stronger plant–plant interactions and increase more when resource availability is high and stable, which allows faster plant growth.

These hypotheses are tested in a pot experiment using two willow (*Salix*) species grown in monocultures or mixtures. This new experiment builds on previous experiences focusing on nutrient limitation (Hoeber et al., 2017), by extending the design to subject plants to water stress and lengthening the study period to ensure more intense plant–plant interactions. In particular and different from most previous studies on diversity effects, we manipulated the frequency and individual amounts of water additions simultaneously, while maintaining the same water total over the growing season to mimic the increasingly intermittent rainfall regimes expected with climate change (IPCC, 2021). The choice of willow species is motivated by their high productivity and frequent use in short-rotation coppice systems (Bonosi et al., 2013; Isebrands & Richardson, 2014; Weih, 2004). Willow mixtures are already successfully used to improve yields in pathogen-prone areas (e.g., leaf rust) (McCracken et al., 2011), but not yet to buffer climatic and resource variability. Willows are thus suitable model species to address our hypotheses thanks to their large phenotypic (i.e., trait) variability without large phylogenetic differences and their relevance for bioenergy production, which in turn has large economic, environmental, and societal implications (Weih et al., 2019). For example, substantial variability among willow species and varieties has been found for resource use efficiency traits such as nitrogen uptake and utilization efficiency (Weih et al., 2021) or the plasticity of biomass allocation to roots versus shoots in response to a greater availability of soil resources (Hoeber et al., 2017). In mixtures of species or varieties with contrasting expressions of resource use efficiency traits, like the ones mentioned above, we expect to see complementarity effects especially along resource availability gradients.

2 | MATERIALS AND METHODS

2.1 | Experimental setup

The experiment has a full factorial design aiming to test the compound effects of species composition, watering, and nutrient addition in an idealized and fully controlled setting. Two *Salix* species were used: *Salix dasyclados* Wimm. var. ‘Loden’ and *Salix schwerinii* E. Wolf. x *S. viminalis* L. var. ‘Tora’. These two species were selected for their different traits: Loden has broad leaves and an architecture with more branches, whereas Tora has narrow leaves and fewer branches (Weih & Nordh, 2002). Tora has lower nitrogen (N) uptake efficiency but a canopy and leaf architecture allowing higher N utilization efficiency than Loden (Weih et al., 2021); moreover, Tora has a stronger plasticity than Loden in reducing root biomass allocation (and increasing shoot biomass allocation) when soil resources are abundant (Hoeber et al., 2017). Six cuttings were planted in each pot, and each

treatment was replicated six times. One third of the containers was planted with cuttings from only Loden, one third with only Tora, and one third with an equal mixture of the two. Planting was done on 22 June 2018 and harvesting of the plants was done in September and October 2019, when plants had stopped growing in height.

Each pot received the same amount of water (1.2 L in 2018/1.6 L in 2019) per week but in two different frequencies. Watering was done twice a week for the high-frequency treatment (W+: 0.6 L in 2018/0.8 L in 2019 each watering), whereas in the low-frequency treatment, watering was done only once a week (W–: 1.2 L in 2018/1.6 L in 2019 each watering). These water addition rates were designed to approximately match potential evapotranspiration in the area, and the watering frequencies allowed establishing dry periods of different duration, followed by contrasting rewetting intensities (W+: short dry period and mild rewetting; W–: long dry period and intense rewetting). To avoid excessive mortality during the warmest periods, in the W+ and W– treatments, the weekly amount of water was, respectively, increased to 0.8 and 1.6 L in 2018 and to 1 and 2 L in 2019 (Figure 1a). Percolation was generally small.

In the water, we added nutrients (Blomstra) at two different concentrations (high N+ and low N–). The nutrient solution ‘Blomstra’ from Cederroth, Upplands Väsby (Sweden), contains NH_4^+ and NO_3^- nitrogen in proportion 19:32 and N, P, K, and Mg in the proportions 50:10:45:3. In the low-nutrient solution, we mixed 10 ml of Blomstra with 51 L of water (0.2 ml/L); in the high-nutrient solution, we mixed 100 ml of Blomstra with 85 L of water (1.2 ml/L). To summarize, there were three species compositions (Loden in monoculture, Loden and Tora in mixture, and Tora in monoculture), two watering frequencies (W+ and W–), and two nutrient concentrations (N+ and N–), amounting to $3 \times 2 \times 2 = 12$ combinations, each in six replicates, with $6 \times 12 = 72$ pots in total.

Before planting, the cuttings were soaked in water to promote growth. The cutting length was 5 cm, and diameters varied between 0.8 and 1.2 cm. Each pot (1.2 kg when empty) contained 13-kg washed quartz sand (Specialsand 0.17 mm, Råda sand AB, Lidköping, Sweden) with a bulk density of 1.4 g m^{-3} , resulting in a soil volume of approximately 9.5 L. The sand was mixed with a small amount of mycorrhiza inoculum obtained from nearby *Salix* plantations. The inoculum added mycorrhiza to the clean sand, to provide the plants with a more natural soil environment without introducing uncontrolled amounts of nutrients. The cuttings were planted at equal distances in each container, in two rows with three cuttings each. In the containers with mixed composition, the first row was planted with Loden, Tora, and Loden and the second with Tora, Loden, and Tora. This pattern ensures that species were homogeneously distributed and that the same proportion of the two species was located at the container edges and corners.

The pots were placed in a net cage under a transparent roof at the SLU Ultuna campus (Uppsala, Sweden), which allowed us to control the amount of added water, and temperature and light fluctuated following the natural variability. Pots were rotated to avoid spurious effects due to the specific location of the pots. The experiment lasted from May 2018 to September 2019. During the winter 2018–2019,

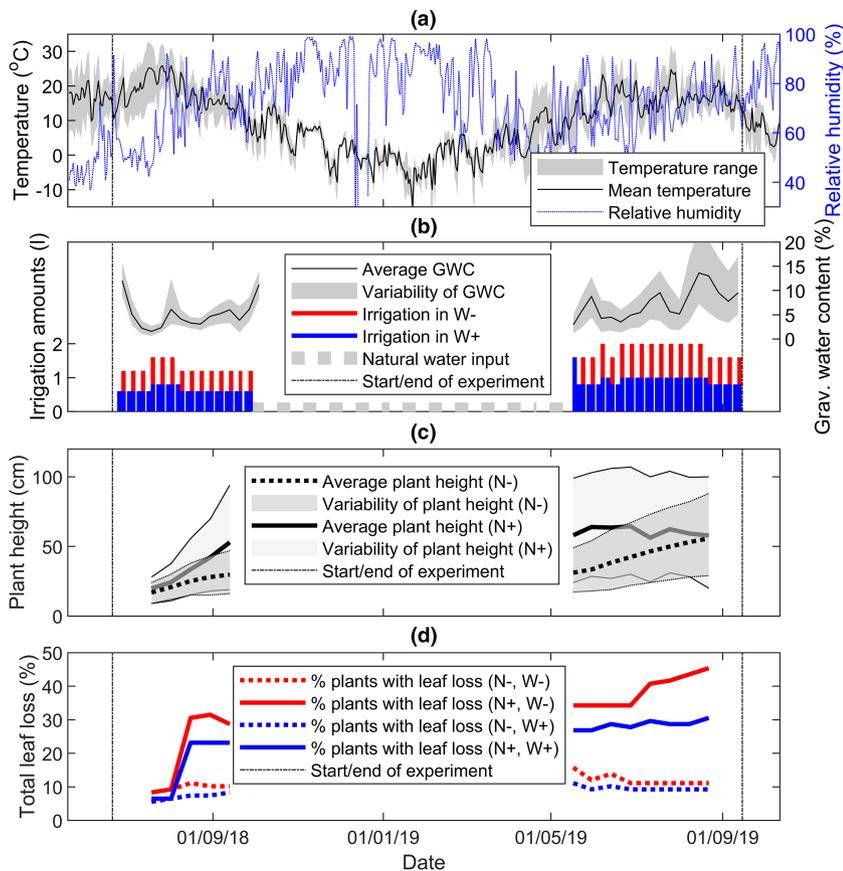


FIGURE 1 Weather data, soil water content, and irrigation, as well as plant growth and drought damage during 2018 and 2019: (a) daily mean and range of air temperature and mean relative humidity; (b) irrigation schedule in the frequent (W+) and infrequent (W-) watering treatments and mean and variability (10th and 90th percentiles) of the gravimetric water content (GWC) across all treatments (GWC were measured before watering, so they reflect the lowest soil water contents at the end of each dry period); (c) mean and variability (10th and 90th percentiles) of plant height in the low nutrient (N-) and high nutrient (N+) treatments; (d) percentage of plants with total leaf loss due to water stress in all combinations of nutrient and water treatments (decreases in per cent damage indicate plants resprouting after the dry periods). No soil water content or plant growth data were collected during the 2018–2019 winter season, so the central parts of (b)–(d) where the legends are placed do not contain any data

the pots were moved to a net cage without roof to avoid desiccation of the plants and placed on the ground with surrounding bags of soil to avoid frost damage. Soil temperature in the containers reached -9°C only in one cold spell and, otherwise, remained mostly above -5°C . These temperatures are commonly experienced by *Salix* plantations in the area and caused only limited damage (Figure 1).

2.2 | Soil water content and plant sampling

We weighed the pots once per week before watering during both growing seasons (May–September) to monitor changes in water content (plant mass was too small to significantly contribute to the weight changes). Plant height and stem diameter were also regularly measured to quantify plant growth rates during the growing seasons. Height was measured during both growing seasons in 2018 and 2019, and diameters were only measured in 2019 because plants were too small in 2018 to ensure accurate measurements. Plant height was measured from the soil surface to the top green leaf base. When plants had multiple shoots, the highest shoot was measured. Stem diameter was measured at the container top, roughly 10 cm over the soil surface. This was mainly done for practical reasons and to avoid measuring the diameter of the cuttings. The height was measured with a folding rule, and the diameter was measured with a calliper.

By the end of the second growing season (2019), all plants were harvested, and dry masses of leaves, stems, and roots were measured.

Nitrogen contents in these different organs were analysed (note that we use the term ‘content’ to indicate mass of N per unit dry weight of tissue; following McNaught & Wilkinson, 1997). Roots of the two species growing in mixtures could not be separated, so that the total root weight per pot was determined. All other measures were conducted on individual plants and averaged or aggregated at the pot level for statistical analysis. Data are presented both on a pot basis and on a plant basis by dividing pot averages or totals by the number of living plants. Allometric relations were instead evaluated at the individual plant level. Even after washing roots carefully, some sand remained, but root weights were corrected for sand content. The mean sand mass fraction of the root samples was estimated at 18% by burning dry roots from eight samples at 500°C for 2 h and then calculating the mass difference before and after burning. This resulted in a correction factor of 0.82 g organic matter per g root sample (standard deviation: 0.12) that we applied to all the root dry weight estimates. Additionally, leaf area and leaf weight of nine leaves from each plant were measured to estimate the specific leaf area (SLA). For this analysis, we selected only fully developed leaves on top of the plants, avoiding leaves that were wilted or damaged.

The first summer of the experiment (2018) was characterized by extreme high temperatures for the area (Figure 1a), and many plants lost all their leaves during the temperature peaks despite additional watering. Dead leaves were collected after a water stress event only in summer 2019 to estimate drought damages and account for leaf loss when calculating the leaf biomass at the end of the experiment.

Total leaf loss was recorded when all leaves had dried. However, plants that resprouted after the end of a dry period were counted as alive in the remaining time of the experiment. Plant survival is thus expressed as percentage of plants in any given pot that retained live leaves at harvest in 2019.

In the analysis of plant biomass components (Section 2.3), leaf, stem, and root weights of the live plants were used. In the estimation of plant diversity effects (Section 2.4), the stem weights of both live and dead plants at harvest were used. These choices are motivated by our intention to interpret stem mass as a measure of primary productivity, including biomass of plants that had died by the time of harvest.

2.3 | Analysis of biomass and nitrogen content data

Plant survival data at the pot scale were compared across treatments using a generalized linear model in Matlab (R2020a, The MathWorks, Inc.; function *fitglm* using a logit link function). The results of this model were also compared with those obtained with a bootstrap method, in which mean survival and its confidence intervals were calculated after resampling for 50 times the observed survival in each treatment group. Mean survival and confidence intervals from this method were used to illustrate the survival data in Figure 2.

Plant trait and biomass data were compared across treatments using four-way analysis of variance (ANOVA) (Matlab function *anovan*), followed by multiple comparison using the Tukey–Kramer

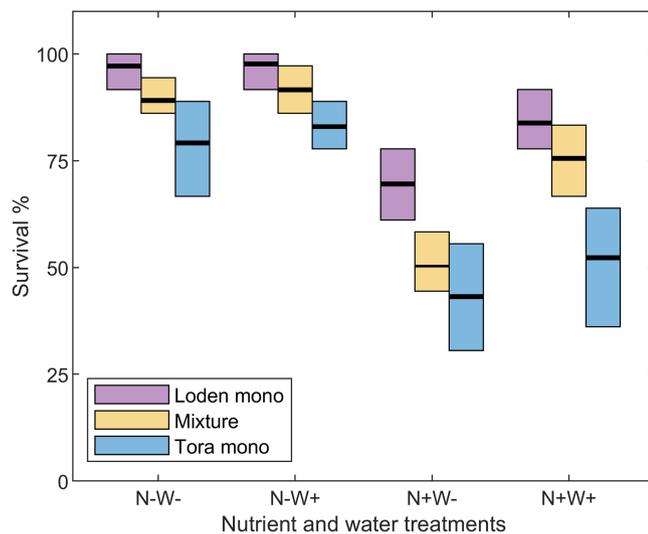


FIGURE 2 Percentage of surviving plants per pot at harvest (2019) as a function of nutrient (high N+ and low N-) and water treatments (frequent W+ and infrequent W-) and species (in each treatment group, left to right: Loden in monoculture, mixture, and Tora in monoculture). Box plots show the mean survival percentages and the 95% confidence intervals of the mean based on bootstrapping. Results from a generalized linear model testing for the treatment effect are reported in the main text. See also the seasonal progression of mortality in Figure 1d

test (function *multcompare*). Water and nutrient treatments and species were regarded as categorical variables and included all combinations of W+/W- (denoted as factor 'W'), N+/N- ('N'), monoculture/mixture ('M'), and Tora/Loden (species, 'S'). In the analysis of root mass, the treatments 'S' and 'M' were merged, and comparisons were made between Tora in monoculture, Loden in monoculture, and Tora and Loden in mixture. In the analyses including time as predictor (plant height-to-diameter ratios; plant diversity effects on stem mass, see Section 2.4), we used a linear model (function *fitlm*), in which treatments and time were accounted for as categorical predictors. These ANOVA and linear regression analyses tested for significant effects of treatments on plant trait and biomass but without considering the abundance of each species as predictor. To account for variable abundance across treatments, the biomass data were also analysed using a more complex linear model, described in Section 2.4.

We calculated ratios of height over diameter as a metric to characterize growth patterns and compared this ratio across treatments and through time (13 June, 13 July, and 8 August 2019) using a linear model. Categorical predictors included the nutrient treatment (0 for N- vs. 1 for N+), watering frequency treatment (0 for W- vs. 1 for W+), species (0 for Tora vs. 1 for Loden), and diversity (0 for monoculture vs. 1 for mixture). Time was treated as a continuous variable (using the days of the year [DOY] for the height and diameter measurement times: 164, 194, and 220).

To compare plant diversity effects between the two seasons, we used stem mass data from 2019 (final harvest) and estimates of stem mass at the end of 2018 based on the allometric relations between stem mass and plant height. These allometric relations were constructed by merging our measurements of plant height (measured throughout 2018 and 2019) and stem mass (measured only in 2019) to those from Hoeber et al. (2017). We did not use data from our N+ treatment because water stress damage reduced plant height due to desiccation of some shoot tips, resulting in high stem mass in relatively short plants by the end of the 2019 growing season. Had we included these data, the estimated stem biomass in 2018 would be overpredicted, because plants in the N+ treatment in 2018 has similar height as in 2019, but lower biomass. After log transforming the stem mass data, we tested both standardized major axis and standard linear regressions (SLR) of stem mass versus height and found no notable difference between the two approaches. Because our goal was to predict stem mass from stem height, we selected standard linear regressions and determined the allometric relations at the species level (Figure S1).

Throughout the results, we report differences and trends when significant ($p < 0.05$).

2.4 | Plant abundance and diversity effects

We first evaluated how the abundance of each species and their interactions—as a first measure of diversity effects—affect leaf, stem, and root mass at the harvest in 2019. As a second step to characterize the role of plant diversity, the net diversity effects on stem biomass in

2018 and 2019 (as a measure of net aboveground primary productivity) were partitioned into complementarity and selection effects following Loreau and Hector (2001).

With the first approach, the role of species abundances was captured by a linear model (Kirwan et al., 2009):

$$B = \beta_L P_L + \beta_T P_T + \delta_{L,T} P_L P_T + \alpha_N N + \alpha_W W + \varepsilon, \quad (1)$$

where B is the mass at pot level of leaf, stem, or roots (or total plant mass); β , δ , and α are the coefficients of the linear regression; P are the plant abundances (number of live plants per pot); N and W are categorical variables indicating the nutrient and watering treatment levels, respectively (with numerical values of 0 and 1, respectively, for low and high nutrient additions and low and high watering frequencies); subscripts L and T indicate coefficients and abundances of Loden and Tora; subscripts N and W indicate coefficients and predictors for nutrient treatment and water treatment; and ε is the residual error. Note that compared with the model described by Kirwan et al. (2009), we did not include explicitly the initial community abundance, because it was the same for all treatments (six plants per pot). Positive interactions as indicated by $\delta_{L,T}$ significantly larger than zero would suggest net positive effects of diversity that are distinct from the contributions of plant abundances (the first two additive terms on the right-hand side of the equation) and treatments. The model in Equation 1 was implemented using the Matlab function *fitlm*. After performing the linear regression of Equation 1, we considered a more complete model including second-order interactions terms and selected the significant terms ($p < 0.05$) using a stepwise regression (*stepwiselm* function).

Next, using the estimated stem mass data in 2018 and those measured in 2019, complementarity, selection, and net effects were calculated following Loreau and Hector (2001):

$$\underbrace{\Delta Y}_{\text{net effects}} = \underbrace{N \overline{\Delta RY M}}_{\text{complementarity effects}} + \underbrace{N \text{cov}(\Delta RY, M)}_{\text{selection effects}}, \quad (2)$$

where ΔY is the deviation from the total stem mass expected in the mixture based on the proportion of the two species, ΔRY is the deviation from the relative stem mass expected in the mixture (i.e., mass in mixture over mass in monoculture) for a given species, M is the mass in monoculture for a given species, the overbars indicate averaging across species, 'cov' is the covariance of ΔRY and M , and $N = 2$ (number of species). In this calculation, only plants with a measurable stem at the end of 2018 or 2019 were considered (including recently died ones, as explained in Section 2.2), resulting in a range of Loden and Tora fractions in the mixtures (at least one individual of each species was always present).

This method requires combining productivity data (in our case stem biomass) from monocultures and mixtures. Because we had six replicate pots per treatment, there are multiple possible combinations of monoculture and mixture pots. Therefore, we randomly selected monoculture and mixture pots and calculated complementarity and

selection effects for each permutation. The procedure was repeated 50 times, resulting in a distribution of values for both complementarity and selection effects. The sum of the complementarity and selection effects (i.e., the net effects) is instead independent of permutation because it is calculated from the total stem mass in all replicates. The complementarity and selection effect datasets were then analysed with linear models including time and treatments as predictors.

By construction, diversity effects scale with the average plant biomass, so that larger effects are expected in systems with larger biomass values. To compare the strength of the diversity effects across treatments or years in which biomass changes significantly, it is thus useful to normalize the diversity effects calculated after Loreau and Hector (2001) by the mean stem biomass of all monoculture pots in the respective treatment and year (as in Craven et al., 2016). We report results for the normalized diversity effects in the main text and for the original calculations in the supplementary information.

Finally, to test the robustness of the analysis based on Loreau and Hector (2001), we also used a similar model as in Equation 1 to predict stem biomass but also including time as predictor, as well as second-order interactions between all predictors. The model was fit with a stepwise regression procedure to isolate significant effects, as described above (in particular testing if $\delta_{L,T}$ was different from zero, capturing significant diversity effects).

3 | RESULTS

3.1 | Soil water content and water stress

The growing season average soil water content (measured before watering) was higher in the frequently irrigated pots compared with the infrequently irrigated (Figure S2 and Table S1) and notably lower in the fertilized pots in 2018 (Figure S2A). In both growing seasons and despite additional irrigation, the soil water content decreased during the warmer periods—one extreme warm period in summer 2018 and one moderately warm in summer 2019 (Figure 1b).

As a result of these conditions and despite our watering (designed to match average evapotranspiration rates for the area, not evapotranspiration in the warmer periods), water stress ensued, leading to complete leaf loss in almost 30% of the individuals in the high nutrient treatment by the end of the summer 2018 (Figure 1d). Many of those plants did not resprout in the following year, resulting in 62% survival at harvest in 2019 (Figure 2). In contrast, 90% of plants survived to the end of the experiment in the low nutrient treatment (significantly higher than in the high nutrient treatment, $p < 10^{-4}$). Survival was lower under low-frequency watering compared with high-frequency watering ($p < 0.05$; Figure 2). Moreover, compared with the mixtures, Tora grown in monoculture had lower survival and Loden had higher survival under any treatment ($p < 0.05$; Figure 2); therefore, survival in the mixtures was intermediate between those in the monocultures.

3.2 | Growth and allometry

During the first summer (2018), plant height increased approximately in a linear way, except for plants categorized as damaged by water stress (Figures 1c and S3). As expected, plant growth in the high nutrient treatment was faster than in the low nutrient treatment, in terms of both absolute and relative growth rates (Table 1). The rates of increase in height differed depending on species, with Tora growing generally faster than Loden and responding to nutrient addition more strongly than Loden (highly significant nutrient–species interaction; Table 1). Species growing in mixtures had similar height increments as those in monoculture (no significant mixture effect).

The majority of plants that had survived water stress in 2018 was still alive in the second growing season (2019), when temperatures were lower than the previous year (Figure 1a). The overall larger plants in 2019 had also more variable height than in 2018. Although plants in the low nutrient treatment continued to grow almost linearly, plants in the high nutrient treatment attained a relatively stable height (Figure 1c), though their diameters continued to increase (Figure S4). In 2019, Tora continued to grow faster than Loden only in the N–treatments. However, Tora plants that had grown the most in 2018 in the high nutrient treatment also suffered the most during warm periods in 2019, resulting in lower growth rate compared with Loden plants in either nutrient treatments (highly significant nutrient–water treatment interactions in 2019; Table 1). In 2019 and under high nutrient availability, the trend in plant height was negative due to water stress damage causing desiccation of the terminal leaves. In both years, the height growth rate in the Loden–Tora mixtures was comparable with those in the monocultures (Table 1).

TABLE 1 Levels of significance and fractions of treatment variance (SS_{tr}/SS_{tot}) from ANOVA analyses comparing growth rate (change in height per unit time) and relative growth rate (change in height per unit time normalized by the initial height) per pot across treatments, considering separately the growing seasons of 2018 and 2019 (see also Figure S3)

Factor	Growth rate		Relative growth rate	
	2018	2019	2018	2019
N	<10 ⁻⁴	<10 ⁻⁴	<10 ⁻⁴	<10 ⁻⁴
W	NS	<0.05	NS	NS
M	NS	NS	NS	NS
S	<10 ⁻⁴	NS	<10 ⁻⁴	<0.01
N*W	NS	<10 ⁻³	NS	<10 ⁻⁴
N*M	NS	NS	NS	NS
N*S	<10 ⁻⁴	<10 ⁻⁴	<10 ⁻³	<0.01
W*M	NS	NS	NS	NS
W*S	NS	<0.05	NS	<0.05
M*S	<0.01	<0.05	<0.05	NS
SS_{tr}/SS_{tot}	0.76	0.63	0.63	0.71

Abbreviations: M, mixture; N, nutrient treatment, NS, not significant; S, species; W, water treatment.

Plant diameter growth was not as heavily impacted by water stress as plant height in the second growing season, because during warm and dry periods, the plants mainly lost leaves at the top of the canopy thereby keeping height stable while diameters kept increasing. Overall, diameters and heights had similar growth trends, due to the clear linear relation between log-transformed height and diameter, at least when nutrient additions were low (Figure S4). Because leaf loss was common in the high nutrient treatments, the linear relation is less obvious there, especially later in the growing season (Figure S4C,F). Consequently, the height-to-diameter ratio was higher in the low nutrient compared with the high nutrient treatment, whereas the water treatment had no significant effect (Table 2). The species allometries differed significantly, and Tora had the highest height-to-diameter ratio.

3.3 | Biomass and allocation

Plants of both species and at both diversity levels grew overall more in the high nutrient compared with the low nutrient treatment (Figure 3a and Table 3). The water and nutrient treatments had a strong interactive effect, such that at low nutrient availability, total biomass was lower under frequent watering, whereas at high nutrient availability, total biomass was higher under frequent watering. Total N accumulated in biomass followed similar patterns as total biomass (Figure 3b and Table 3), but in addition to the strong nutrient and nutrient–water interaction effects, we also found clear species effects (Loden accumulated more N than Tora) and nutrient–species interactions (Loden accumulated disproportionately more N relative to Tora in the high nutrient treatment).

The fractional biomass allocation to roots was higher, whereas allocation to leaf and stem was lower in the low nutrient treatment (Figure 4). The same pattern occurred when comparing absolute root, leaf, and stem biomass between nutrient treatments, regardless of whether biomass was expressed on a per plant or per pot basis

TABLE 2 Parameters of the linear model relating the ratio of stem height to diameter (expressed in cm/mm) to time (DOY in 2019), nutrient addition treatment (0 for N– and 1 for N+), watering treatment (0 for W– and 1 for W+), number of species (0 for monoculture and 1 for mixture), and species identity (0 for Loden and 1 for Tora; see also Figure S4)

Effect	Parameters	p-value
Intercept	8.79	<10 ⁻⁴
Time (DOY)	0.00939	<10 ⁻³
N	–2.45	<10 ⁻⁴
W	0.0204	NS
M	0.0397	NS
S	2.68	<10 ⁻⁴

Note: All treatment levels are regarded as categorical variables, expressed as 0 or 1. Overall fraction of explained variance: 0.46. Abbreviations: DOY, day of year; NS, not significant.

(Figure S6 and Tables 3 and 4). When aggregating biomass at the pot level, Tora grown in monoculture had higher stem but lower leaf and root biomass compared with Loden (Figure 5). The water treatment affected the stem and root biomass per pot, which were, respectively, lower and higher in the high watering frequency treatment compared with the low-frequency treatment (Figure 5 and Table 3). Moreover, we found significant interactive effects of nutrient and water treatments on stem and root biomass (Figure 5 and Table 3). In fact, the biomass of roots and stems of plants receiving water frequently was

higher than that receiving water infrequently in the high nutrient treatment but lower in the low nutrient treatment (Figure 4).

Patterns in biomass per plant were overall similar to the patterns noted at the pot scale but with fewer significant effects due to the higher variability of measurements at the plant level (Figures S5 and S6). In particular, the nutrient–water interaction effect was significant only for total biomass, but not for total N per plant. However, some differences between species emerged—for example, Tora accumulated more biomass per plant in the stem than Loden, when grown both in monocultures and in mixtures (Figure S6). Interestingly, growing in mixtures compared with monocultures had no significant direct or interactive effect on any biomass compartment when evaluated on a per plant basis (Table 4).

Accounting for plant abundance with the linear model of Equation 1 led to similar results (Table S3). Abundances had generally positive effects on total biomass and biomass components. Leaf, stem, and total biomass were higher in the high nutrient treatment, and root

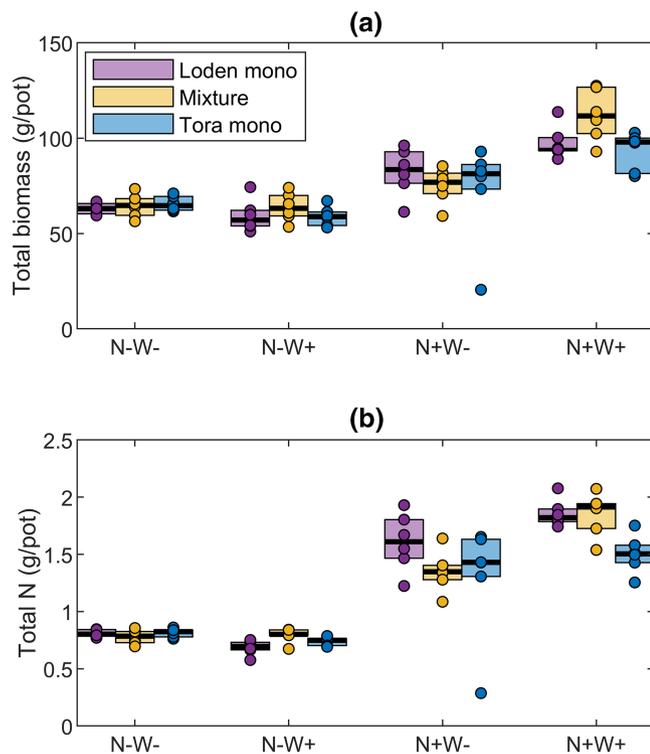


FIGURE 3 Effect of nutrient (high N+ and low N−) and water (frequent W+ and infrequent W−) treatments on (a) total plant biomass and (b) total plant nitrogen per pot at harvest (2019). Box plots show median, quartiles, and data points. Results from ANOVA analysis testing for treatment effects are reported in Table 3 and data on a per plant basis in Figure S5

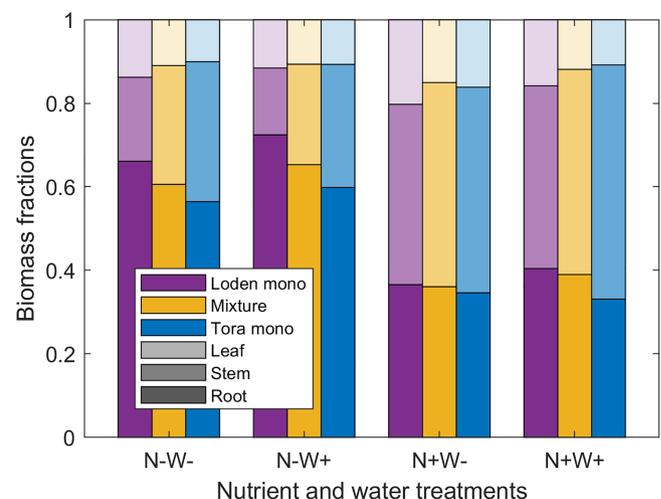


FIGURE 4 Biomass allocation at harvest (2019) per pot, expressed as fractions of leaf, stem, and root biomass (lighter to darker shades) across nutrient (high N+ and low N−) and water (frequent W+ and infrequent W−) treatments, for the two *Salix* species grown in monocultures and in the mixture

TABLE 3 Levels of significance and fractions of treatment variance (SS_{tr}/SS_{tot}) from ANOVA analyses comparing total, leaf, stem, and root biomass per pot and average root N contents per pot across treatments

Factor	Total biomass per pot	Leaf biomass per pot	Stem biomass per pot	Root biomass per pot	Total N per pot	Pot-average root N content
N	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$
W	$<10^{-3}$	NS	$<10^{-3}$	$<10^{-4}$	$<10^{-2}$	NS
S	NS	$<10^{-3}$	$<10^{-3}$	$<10^{-4}$	$<10^{-3}$	<0.05
N*W	$<10^{-4}$	NS	$<10^{-4}$	$<10^{-3}$	$<10^{-2}$	NS
N*S	NS	<0.1	NS	NS	$<10^{-2}$	$<10^{-3}$
W*S	NS	NS	NS	<0.05	NS	NS
SS_{tr}/SS_{tot}	0.71	0.64	0.86	0.60	0.90	0.95

Abbreviations: N, nutrient treatment; NS, not significant; S, species (Loden in monoculture, Tora in monoculture, and mixture); W, water treatment.

TABLE 4 Levels of significance and fractions of treatment variance (SS_{tr}/SS_{tot}) from ANOVA analyses comparing biomass per plant and N contents of leaves and stems, as well as specific leaf area across treatments

Factor	Leaf biomass per plant	Stem biomass per plant	Leaf N content	Stem N content	Specific leaf area
N	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$
W	NS	NS	NS	NS	$<10^{-4}$
M	NS	NS	NS	NS	NS
S	NS	$<10^{-4}$	<0.05	$<10^{-4}$	NS
N*W	NS	<0.05	NS	NS	$<10^{-2}$
N*M	NS	NS	NS	NS	NS
N*S	NS	<0.01	NS	NS	NS
W*M	NS	NS	NS	NS	NS
W*S	NS	<0.1	NS	NS	NS
M*S	NS	NS	NS	NS	NS
SS_{tr}/SS_{tot}	0.38	0.66	0.83	0.79	0.87

Abbreviations: M, mixture, N, nutrient treatment, NS, not significant; S, species; W, water treatment.

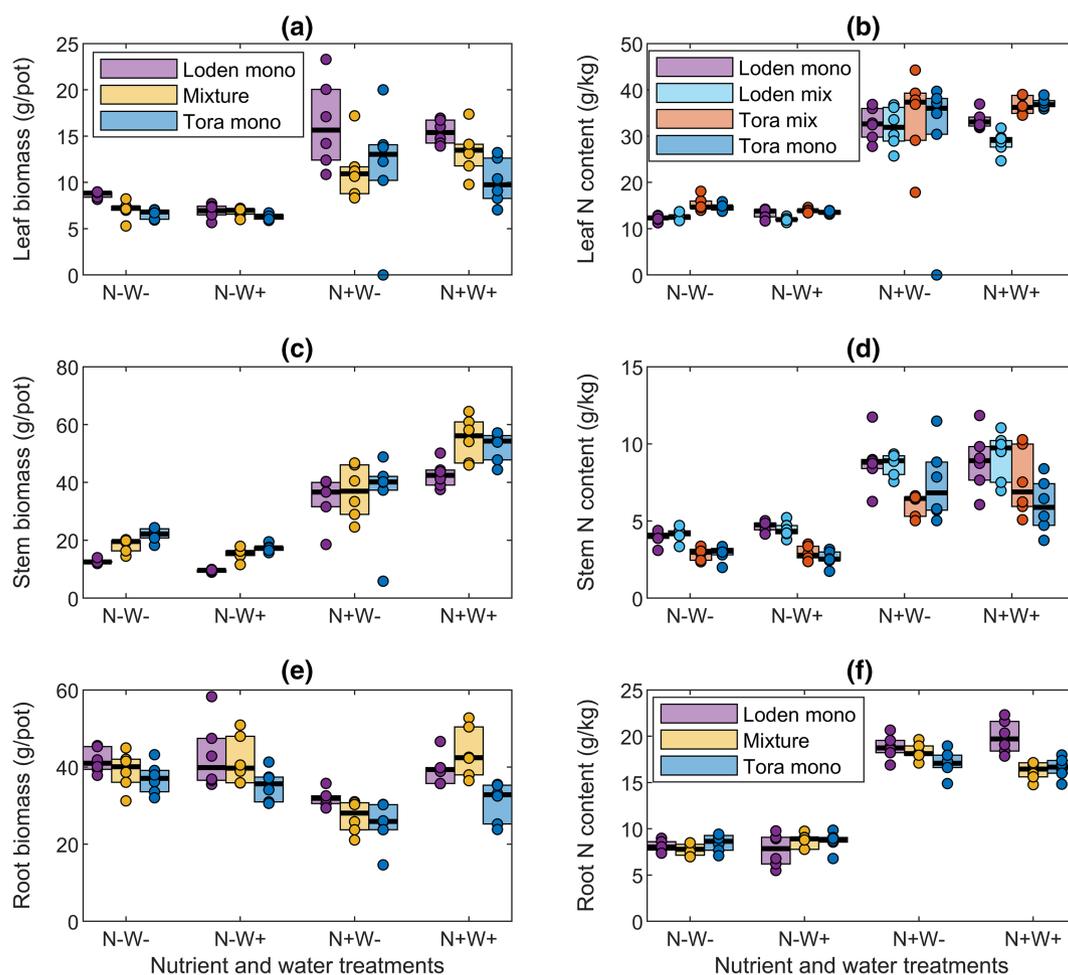


FIGURE 5 Effect of nutrient (high N+ and low N-) and water (frequent W+ and infrequent W-) treatments on (a) leaf, (c) stem, and (e) root biomass per pot at harvest (2019) and mean nitrogen content in (b) leaves, (d) stems, and (f) roots. Leaf and stem nitrogen contents are shown separately for Loden and Tora grown in mixtures. Box plots show median, quartiles, and data points. Results from ANOVA analysis testing for treatment effects are reported in Table 3, and data similar to those presented in (a) and (c) are shown on a per plant basis in Figure S6

biomass was higher in the high-frequency watering treatment. When accounting also for interactive effects in the stepwise regression, the interactive effect of nutrient and water treatments was not significant for stem and root biomass (as it was in the ANOVA analysis; see Table 3), whereas the interactive effect on total biomass was positive.

3.4 | Nitrogen contents and SLA

Nitrogen (N) contents were higher in the high nutrient treatment for all plant organs, with leaves having higher N contents than roots and stems (Figure 5b,d,f and Tables 3 and 4). N contents also varied depending on species, with higher leaf N contents in Tora and higher stem N contents in Loden. Consistent with the N content measurements, SLA was higher in the high nutrient treatment (Table 3 and Figure S6C). Moreover, SLA was lower in the frequently irrigated treatment and was affected by a positive interaction between the nutrient and water treatments. Root N was significantly affected by species but with an interactive effect such that root N content was higher in Loden under high nutrient availability and in Tora under low nutrient availability. No significant leaf or stem N content differences between monoculture and mixtures were found.

3.5 | Diversity effects

Diversity effects were calculated following the method by Loreau and Hector (2001) (Equation 2) using stem biomass data only, in both original (not normalized) and normalized forms, but here, we focus on the normalized values, which remove the effect of average biomass in the calculation (Figure 6; the original diversity effects are shown in Figure S7). We have also calculated the diversity effects based on the initial abundance of the respective *Salix* species, but results were similar, so in this section, we only report diversity effects calculated with the actual abundance data.

The normalized complementarity effects were positive, except in the low nutrient, low water frequency treatment (Figure 6a). The normalized selection effects were positive in the low nutrient treatment and negative in the high nutrient treatment in 2018, turning neutral or positive in 2019 (Figure 6b). As a result, the normalized net effects were positive (Figure 6c). Time had a positive effect on normalized selection and a negative effect on normalized complementarity effects (Table 5), resulting in generally increasing net diversity from 2018 to 2019 (Figure 6c). However, the contribution of time was not as strong as that of the nutrient and water treatments. High watering frequency and nutrient addition increased complementarity and decreased selection effects (Table 5). The original (not normalized) diversity effects followed similar patterns as the respective normalized values, but complementarity was not affected by time (Table 5 and Figure S7).

Diversity effects in 2019 calculated using the linear model of Equation 1 were positive only for root biomass ($\delta_{L,T} > 0$), whereas they were positive but not significant for stem or total biomass (Table S3). When including time as predictor, we also found a positive interaction between nutrient availability and $P_L P_T$ (Table S4), indicating that diversity effects were more positive in the high nutrient treatment, consistent with the results from the Loreau and Hector (2001) method.

4 | DISCUSSION

4.1 | Biomass allocation and morphological traits (HP1)

Plant growth is promoted by nutrient and water availability, but plant ontogeny also influences growth (Gedroc et al., 1996). During growth, a plant can adjust the growth rate of its organs within some limits. According to optimal partitioning theory, a plant will allocate resources to the organs acquiring the most limiting resource, although there are constraints to how effectively this can be done due to mechanical and metabolic limitations (McCarthy & Enquist, 2007).

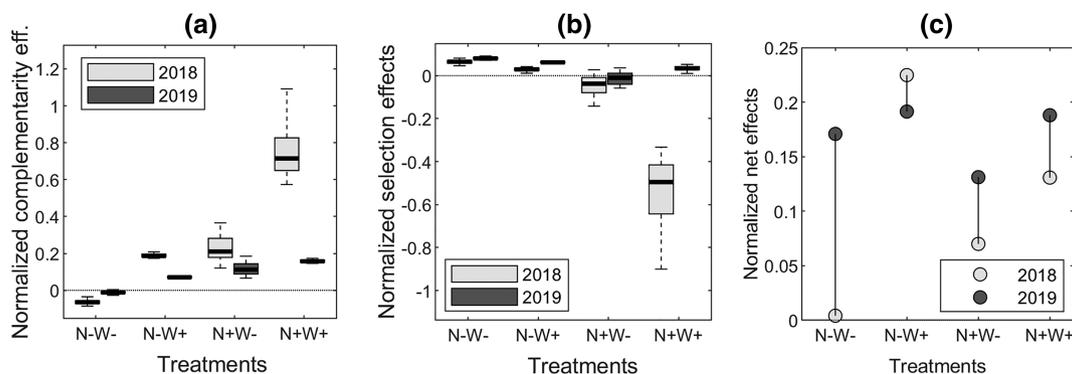


FIGURE 6 Normalized diversity effects under different nutrient (high N+ and low N-) and water (frequent W+ and infrequent W-) treatments, based on estimated stem mass in 2018 and 2019: (a) complementarity effects, (b) selection effects, and (c) net effects. Normalized diversity effects are calculated for 50 permutations of monocultures and mixture pairs. Box plots show median, quartiles, and range after excluding outliers. Results from a linear model of complementarity and selection effects testing for treatment effects are reported in Table 5 and the original (not normalized) diversity effect data are shown in Figure S7

TABLE 5 Parameters and fractions of explained variance (R^2) of the linear models relating the original and normalized complementarity and selection effects to time (year), nutrient addition treatment (0 for N– and 1 for N+), and watering treatment (0 for W– and 1 for W+)

	Original (not normalized) diversity effects				Normalized diversity effects			
	Complementarity ($R^2 = 0.84$)		Selection ($R^2 = 0.46$)		Complementarity ($R^2 = 0.67$)		Selection ($R^2 = 0.49$)	
	Parameters	<i>p</i> -value	Parameters	<i>p</i> -value	Parameters	<i>p</i> -value	Parameters	<i>p</i> -value
Intercept	–1.27	$<10^{-4}$	–0.0257	NS	0.0308	<0.05	0.0402	<0.01
Time (year)	0.149	NS	2.59	$<10^{-4}$	–0.196	$<10^{-4}$	0.168	$<10^{-4}$
N	5.78	$<10^{-4}$	–2.21	$<10^{-4}$	0.271	$<10^{-4}$	–0.204	$<10^{-4}$
W	2.98	$<10^{-4}$	–1.25	$<10^{-4}$	0.228	$<10^{-4}$	–0.130	$<10^{-4}$

Note: Diversity effects are calculated for 50 permutations of monocultures and mixture pairs (see also Figure 6). All treatment levels are regarded as categorical variables, expressed as 0 or 1 for convenience of interpretation. Abbreviations: NS, not significant; *p*, level of significance.

This motivated the first part of our first hypothesis (lower nutrient and more intermittent water availability increase allocation to below-ground biomass and to thicker, smaller leaves). We tested this hypothesis by combining nutrient additions to a manipulation of water availability where the frequency and amounts of water applications were simultaneously altered—not the total amount of water added. Although it is well known that total amounts of precipitation shapes plant community productivity and composition (Huxman et al., 2004), the effect of rain intermittency is less known (but see, e.g., Fay et al., 2003). With this design, our aim was to study water stress as a result of longer dry periods between more intense rain events—as expected in a warmer climate (IPCC, 2021).

Compared with the water treatment and the *Salix* community composition, the nutrient treatment had the largest impact in our pot experiment. As expected and previously reported in other diversity experiments (Hoerber et al., 2017; von Felten & Schmid, 2008), plants receiving low nutrient additions grew slower and had higher root-to-shoot ratios than those growing under high nutrient additions (Figures 4 and 5). This result can be explained in the light of previous theories of multiple resource acquisition (Ågren & Franklin, 2003; Rastetter & Shaver, 1992)—less roots in proportion to other plant parts are needed to acquire nutrients at higher soil nutrient contents. Plant traits were also changed by nutrient treatment. With increasing nutrient availability, SLA increased (Table 4 and Figure S6C) and stem height-to-diameter ratio decreased (Table 2). The higher SLA (thinner leaves) is in line with studies showing that plants in nutrient rich environments will increase their SLA to maximize light capture (I. J. Wright et al., 2002). A more unexpected result was the lower SLA (thicker leaves) under high watering frequency (Figure S6C), as we expected thinner leaves from the first part of HP1 and also from experiments reporting the effect of drought on SLA in willows (Weih et al., 2011).

As for nutrient limitation, allocational shifts towards roots in response to drought have also been frequently observed (including among willow species) and can be interpreted as functional adaptations to drought independent of plant size (Bonosi et al., 2010; Poorter & Nagel, 2000; Weih et al., 2011). However, it should be noted that our watering treatment did not alter the total water added but its distribution through time, thus changing the temporal

variability of soil water. Under low watering frequency, dry periods were longer and soil water content at the end of those periods was lower (Figure S2), while after irrigation, it was higher than under high-frequency watering. In a conceptually similar experiment with grassland vegetation, the root-to-shoot ratio increased with more infrequent but larger water additions compared with the control (Fay et al., 2003), whereas we observed a slight decrease in contrast to our HP1 (Figure 4). This discrepancy could be due to the changes in species composition in the study by Fay et al. (2003), which might have caused larger variations in root-to-shoot ratio than in our minimal willow community.

In our study, interactive effects of nutrient and water treatments occurred, supporting the second part of HP1. Plants growing faster under high nutrient availability experienced larger leaf loss during warm and dry periods than under low nutrients. *Salix* spp. are ‘drought avoiders’ (contrary to ‘drought tolerators’; see Savage et al., 2009) and can reduce their leaf area as an adaptation to limit water loss (Manzoni et al., 2015; Munne-Bosch & Alegre, 2004). In our experiment, in addition to leaf loss, mortality was also higher in the high nutrient treatment (Figure 2). This more pronounced water stress was due to higher water consumption by the larger plants in the high nutrient treatment during the warmest periods of the first growing season. Indeed, soil water content was lower in the high nutrient treatment pots than in the low nutrient pots in 2018, but generally higher in 2019, except for Loden in monoculture (Figure S2A). The higher water content in 2019 was likely due to the smaller number of surviving plants in the high nutrient treatment pots, which were using less water overall despite their larger size, compared with plants in the low nutrient treatment. This interaction between water and nutrient treatments had been previously reported in experiments with *Salix* (Weih, 2001), and more in general, drought damages tend to be larger in plant communities previously exposed to favourable conditions for growth (Zhang et al., 2021). This result points to a possible increase in drought vulnerability of previously fast-growing plant communities in nutrient rich conditions when they are exposed to a serious water shortage.

Moreover, the direction of responses to watering frequency of plant allocation differed depending on nutrient addition. When

watering often, we found higher root and stem biomass—and thus overall larger plants—compared with low-frequency watering, but only under high nutrient addition (Figure 5). In contrast, at low nutrient availability, roots had higher biomass in the low-frequency watering treatment. This indicates that plant allocation strategies changed fundamentally due to nutrient conditions, causing them to respond differently to watering frequency. Additional interactive effects of water and nutrient availability might thus be at play, beyond the hypothesized mechanism of larger plants consuming more water. Other studies have shown that the duration of water stress can cause different drought responses; for example, plants grown under permanent water stress acclimate by means of increased investment into roots (Schaff et al., 2002; Wikberg & Ögren, 2004), whereas plants subjected to temporary water stress rapidly adjust to well-watered conditions after the end of drought (Bonosi et al., 2010). In our study, we did not vary the overall water supply, so responses in the water treatment likely reflect short-term adjustments that are not as strong as those observed in the nutrient treatment. Our results thus indicate complex allocation patterns when water stress interacts with nutrient supply, and these patterns cannot be readily explained by current theory.

It is well known that drought stress responses can vary considerably between willow species and genotypes (Weih, 2001; Weih et al., 2006; Wikberg & Ögren, 2004). Also, in this study, the two *Salix* species did not respond in the same way to the water treatment, as Tora plants were more susceptible to water stress than Loden plants (Figure 2). This might appear to contrast with the observation that Loden depleted soil water more than Tora in all nutrient and water treatment combinations in 2018 and still in the high nutrient treatment in 2019 (Figure S2A). However, this pattern can be explained by considering that Tora suffered higher leaf loss during 2018, which in turn reduced transpiration and allowed soil water content to remain higher than for Loden.

4.2 | Diversity effects across treatments (HP2) and through time (HP3)

An open question in ecology is when and if diversity promotes ecosystem functioning via complementary resource use and/or facilitation mechanisms (Isbell et al., 2011; Tilman et al., 1997). Even in our very low diversity experiment, with only two species in the genus *Salix*, we found positive complementarity effects of the mixtures compared with the monocultures for most treatments but most clearly under high watering frequency and high nutrient additions (Figures 6A and S7A). Variations in net diversity effects across treatments were largely driven by these complementarity effects (selection effects were numerically smaller), as also shown in previous studies focusing on either water limitation or nutrient additions (Craven et al., 2016; von Felten & Schmid, 2008). This indicates that *Salix* mixtures not only performed better than would be expected by the mixture composition (positive net effects; Figure 6C) but also that species interactions (complementarity effects) and not the overperformance or underperformance of individual species (selection effects) was driving the improved community-level performance.

Notably, these positive interactions are in contrast to HP2 that complementarity effects would be higher when resource availability is low and/or highly variable. This finding is also in contrast to previous results in a similar but shorter experiment with *Salix* mixtures where complementarity effects were negative at high nutrient availability and positive at low nutrient availability (Hoerber et al., 2017). Similarly, net effects were decreased under fertilization in diversity experiment with grassland species (Craven et al., 2016; von Felten & Schmid, 2008).

It should be noted that diversity effects can only be evaluated when biomass estimates are available for all the species in the mixtures (in addition to the monocultures). Separating the roots of the two *Salix* species was not feasible, so we used stem biomass as a species-specific measure of productivity. In the low nutrient treatment, allocation to stems was lower than in the high nutrient treatment (Figure 4), so the larger diversity effects found in communities growing with ample nutrient supply can in part be explained by allocation patterns—with high nutrient availability, plants grow larger stems, and thus, any diversity effect is amplified. However, compared with the low nutrient treatments, in the high nutrient treatments, allocation to stems is only 1.5 to 2 times higher (for low- and high-frequency watering, respectively), and net diversity effects are 2 to 4 times higher if they are not normalized. This suggests that allocation patterns alone cannot explain the higher diversity effects observed in the fertilized plant communities. Normalizing the diversity effects removes this amplification due to unequal allocation to roots and stems, but we showed that the treatments affected both complementarity and selection in the same way even when considering normalized diversity effects (Table 5). This finding lends support to stronger complementarity under nutrient rich conditions and moderate soil water fluctuations, independent of biomass size per se.

The linear regression of Equation 1 allows the evaluation of diversity effects by examining the significance of the interaction between species abundances (Kirwan et al., 2009). This approach highlighted that diversity effects were generally positive, though significant only for root biomass when considering the 2019 harvest data. Notably, the interaction between nutrient availability and the product of the species abundances was positive when considering both 2018 and 2019 stem mass data, indicating enhanced diversity effects at high nutrient availability. Positive interactions as estimated from these regression models can be conceptually compared with positive net effects (Figure S7C), which, however, are calculated as expected values across all replicates and thus do not include confidence intervals. Therefore, at least the sign of the diversity effects is consistent between these two methods, but a quantitative comparison is hindered by the intrinsic differences in the estimation approaches.

Apart from negative admixing effects on the leaf N contents of field-grown willows, Weih et al. (2021) found little evidence supporting that traits linked to N uptake and use are significantly affected by the diversity level per se. Similar to Weih et al. (2021), we found no clear evidence for mixture effects on traits linked to N uptake and use in the two species used here. Thus, the observed complementarity effects probably cannot be explained by mixture effects

on N utilization because no significant differences were found in N contents when comparing mixtures and monocultures, except possibly in roots (Table 3). There we found significant differences in root mass among Loden in monoculture, Tora in monoculture, and the species mixture, but we could not separate species and mixture effects because the roots of the two species could not be separated. In other words, high complementarity in the mixtures was not associated with higher leaf or stem N contents (Table 4). Similarly, total accumulated N was also not consistently higher in the mixtures (Figure 3). High complementarity did not seem to be associated to ameliorating effects on environmental conditions either (Wright et al., 2021), because soil water content was not always higher in mixtures than in monocultures (Figure S2) and in fact survival in the mixtures was intermediate between survival in the monocultures (Figure 2). One possible explanation could be that plant interactions are simply more intense when plants grow larger.

Net diversity and selection effects increased from the first to the second year, but complementarity effects decreased with time when normalized or were insensitive to time when not normalized (Table 5). This result is inconsistent with our HP3 (complementarity effects increase through time) and results in earlier studies (Cardinale et al., 2007; Tatsumi, 2020). However, in those studies, diversity effects were not normalized by average biomass, so that the observed increases in complementarity might be a result of the overall increasing biomass or a combination of increasing biomass and intensifying plant–plant interactions as plants grow larger through time (Cardinale et al., 2007). This explanation would also be consistent with the higher (not normalized) complementarity found in the high nutrient and high watering frequency treatments, where plant biomass was on average higher. It is also possible that our estimation of stem biomass in 2018 based on species-specific allometric relations between height and biomass biased the results. However, the robustness of these relations (Figure S1) for plants of the size expected in 2018 suggests that this step is less than or as uncertain as other steps in the diversity effect calculations.

It is also likely that pot size was constraining growth more as time progressed (Poorter et al., 2012), in particular in the high nutrient treatment where plants were larger, reaching up to 10 g biomass per litre of rooting volume. This might have two effects: on the one hand, it might decrease opportunities for spatial niche segregation in the soil (e.g., Silvertown et al., 2015), causing a decrease in (normalized) complementarity effects through time for a given nutrient level; on the other hand, it might decrease differences between growth rates between nutrient treatments at a given time, as larger plants are inhibited more. The first effect is not consistent with the higher complementarity in the high nutrient treatment, which we speculate might have been caused by different timing of nutrient acquisition by the two willow species. The second effect could result in more similar biomass or diversity effects between nutrient treatments than would occur with larger pots, because of the possible growth inhibition of the larger plants. However, we already found strong nutrient effects, so that larger pots would likely amplify these fertilization effects.

Although the diversity effects were clearly dependent on treatment and time, we did not identify specific mechanisms driving these patterns (e.g., the nature of plant–plant competition or mutualistic interactions). Future research could therefore focus on these mechanisms and how they shape growth and resource use strategies, thus maintaining and potentially promoting diversity under water or nutrient limited environments.

4.3 | Conclusions

We found that combined water and nutrient limitations interactively shape plant growth and species interactions under resource competition. Low nutrient levels—by constraining growth—allow maintaining less stressful soil water contents and promote stress-survival traits such as increased root to shoot ratio and a decreased SLA (consistent with HP1). This interactive effect suggests that plant communities growing rapidly thanks to abundant resources might suffer the most during droughts. However, it is also possible that this effect was emphasized by the relatively small pot size, which promoted water stress more than would occur in a natural environment with deeper soil.

The productivity in mixtures consisting of the two *Salix* species was higher than expected by averaging the productivity of the two monocultures for all treatments. Complementarity effects were stronger under high nutrient availability and watering frequency (contrary to HP2) and decreased through time when normalized by the average biomass (contrary to HP3). Therefore, plant size rather than time per se appears to control complementarity effects. The positive net diversity effects could not be explained by nitrogen contents in plant stems and leaves, suggesting that interspecific interactions become stronger or differentiation in nutrient acquisition strategies becomes more beneficial as plants grow larger when resources are more available.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors designed the experiment. M. L. conducted the experiment and drafted the manuscript. M. L. and S. M. analysed the data. S. M. and M. W. revised the manuscript. S. H. commented on the manuscript.

DATA AVAILABILITY STATEMENT

The data are shown in the main text figures or in the supplementary data. Pot level data on the number of survived plants and root, stem, and leaf biomass and tissue N contents are available in the Bolin Centre Database (dataset “Salix biomass and nitrogen content measured in a pot experiment, Uppsala, Sweden, 2018–2019,” <https://doi.org/10.17043/manzoni-2021-salix-1>).

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