

Does plant trait plasticity explain the relative competitive ability of plant species along a N:P stoichiometric gradient?

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Abstract

The balance between nitrogen and phosphorus is an important determinant of plant species competition and therefore for species diversity. To see whether the competitive advantage of certain species can be explained by plasticity in plant functional traits a greenhouse experiment was conducted. For this experiment two N-specialists, two P-specialists and two opportunistic species were grown at with five N:P supply ratios (i.e. 1,7; 5; 15; 45; 135) at three overall nutrient supply levels (i.e. low, medium, high).

The intermediate N:P supply ratio and the slightly N-limited treatment have the highest total biomass. Under extreme nutrient limitation all species produce significantly less biomass, performing better under N-limitation than under P-limited conditions. The results indicate that opportunistic species overall have the lowest success ratio, while the overall success ratio of N-specialists is highest.

Plant trait values are significantly affected by nutrient supply level, species and N:P supply ratio. Overall most interactions effects are significant, indicating the importance of inclusion of the effect of nutrient level as well as the N:P ratio. In general all species show plasticity in traits, but not for each trait. Best performing species was *Alopecurus pratensis* (i.e. a N-specialist), for this species phenotypic plasticity was only found for the leaf length. This, as well as the outcome of the regression analysis, indicates that phenotypic plasticity in this selection of traits (i.e. leaf length, SLA and SRL) does not explain the success ratio of a species.

Samenvatting

Naast de beschikbaarheid van stikstof en fosfaat is ook de verhouding tussen deze elementen van invloed op de competitie tussen soorten en dus belangrijk voor de biodiversiteit in een ecosysteem. In dit onderzoek is, door middel van een kasexperiment, bekeken of het competitief voordeel van plantensoorten verklaard kan worden door de mogelijkheid om karakteristieke eigenschappen sterker of minder sterk te uiten. Twee N-specialisten, twee P-specialisten en twee opportunisten zijn onder verschillende omstandigheden ingezet. Deze omstandigheden zijn combinaties tussen vijf verhoudingen tussen stikstof en fosfaat (1,7; 5; 15; 45; 135) en drie hoeveelheden (laag, midden, hoog).

Kijkend naar het effect van de N:P ratio op biomassa laat zien dat de totale biomassa van alle plantensoorten het hoogst is onder gemiddelde tot licht N-gelimiterde omstandigheden. De planten hebben een hogere totale biomassa onder extreme N-limitatie dan onder extreme P-limitatie. De succes ratio van opportunisten is het laagst, terwijl die van N-specialisten het hoogst is.

De hoeveelheid nutriënten en de N:P ratio beïnvloeden de waarden van planteigenschappen. Interactie effecten zijn vrijwel altijd significant, dit laat zien dat het belangrijk is om altijd te kijken naar de effecten van de hoeveelheid nutriënten en de verhouding tussen stikstof en fosfaat. De resultaten laten zien dat alle planten de mate van uiting van planteigenschappen kunnen aanpassen hebben, maar niet elke plantensoort laat dit zien voor elke eigenschap. De soort met de hoogste succes ratio, onder alle omstandigheden, is *Alopecurus pratensis* (dit is een N-specialist), deze soort laat alleen voor de bladlengte plasticiteit zien. Samen met de uitkomsten van de regressie laat dit zien dat plasticiteit van bladlengte, SLA en SRL niet de verklaring is voor het succes van de voor dit onderzoek geselecteerde plantensoorten.

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1.0 Introduction

Plant strategies in space and time are mainly driven by resource supply (i.e. availability of light, water and nutrients) (Ordonez, 2009). In classical theory nitrogen (N) is considered to be the most important nutrient steering plant species composition and richness. An increased level of nitrogen can reduce species richness through favouring species, which are better adapted to high N availability (Stevens et al., 2004). Species richness is the number of species in a given area and is an important component of biodiversity. More recent studies indicate however that not only nitrogen but also phosphorus (P) enrichment is a main driver for the loss of biodiversity in Western Europe (Wassen et al., 2005; Ceulemans et al., 2012).

Besides the availability of a single element the balance between elements is an important determinant of species competition and therefore for species diversity (Güsewell, 2004; Olde Venterink and Güsewell, 2010; Fujita, 2010). This balance is described by the N:P stoichiometry. The average N:P ratio in terrestrial plants is 12-13 (Güsewell, 2004). For plants of a site a high N:P ratio (>16) indicates P-limitation and a low N:P ratio (<13,5) indicates N-limitation, whereas an N:P ratio between 13,5 and 16 indicates N/P co-limitation (Güsewell et al., 2002). Limiting elements are those of which the supply must be increased in order to increase the production (Güsewell, 2004). Plant species which are N-limited obtain maximal production by increasing N supply, while for P-limited species maximal production is obtained by increasing the supply of P.

The type of nutrient limitation and the N:P ratio in the vegetation both correlate with the biomass production of the vegetation. Maximum above ground biomass under P-limitation is much lower than under N-limitation (Olde Venterink, 2011). Differences in productivity might cause the diversity-productivity curve to be narrower under P limitation than under N limitation (Wassen et al., 2005), which indicates that (for the Eurasian wetlands) under intermediate to high productivity higher species richness can be achieved under N than under P limitation (Olde Venterink, 2011). Due to these differences in the diversity-productivity relationship for N- and P-limited conditions a shift from one limiting nutrient to another can have a severe effect on the local species richness (Olde Venterink et al., 2003). Figure 1 shows a conceptual representation of Olde Venterink (2011) where is shown how the species richness-productivity patterns can differ between plant communities which are growth limited by different nutrients (Olde Venterink, 2011).

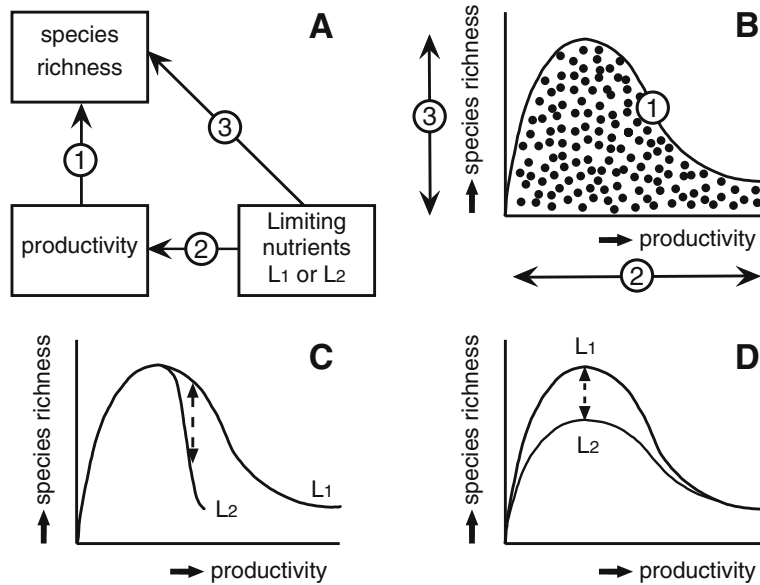


Figure 1: A conceptual representation from Olde Venterink (2011) showing how species richness productivity patterns can vary for between plant communities which are limited by different nutrients (L1, L2). Panel B shows the hump shaped relationship that is observed in field studies, maximum richness and maximum variation in richness is found at intermediate productivity. Panel C shows the possible differentiation in productivity levels for communities which are growth limited by different nutrients, supported with data (Olde Venterink et al., 2003; Wassen et al., 2005). Panel D shows a possible variation in maximum species richness under growth limitation by different nutrients (Olde Venterink, 2011).

The availability of N and P acts as an environmental filter for plant species occurrence (Keddy, 1992). Changing the relative availability of N and P by adding either element induces species-specific responses of plant growth and death rates, nutrient uptake and retention (Fujita et al., 2010b). Fujita et al. (2010b) showed there is an independent effect of N:P supply ratios on plant death rate, apart from the effects of N or P supply levels. Interspecific differences in growth strategy causes some species to be better adapted to N-limited conditions (N-specialists) or P-limited conditions (P-specialists). Opportunistic species use resources for current growth whenever they are available, by this they achieve longer growing periods at the expense of higher risk of tissue damage (Castro-Diez et al., 2003). Opportunists are able to profit from N- and/or P-enrichment. Interspecific differences in species' response to N:P ratios may affect the competition and alter the structure of the community. Species composition under N-limitation compared to P-limited conditions is very different, even when the total productivity of the vegetation is identical (Fujita et al., 2010b; Olde Venterink, 2011).

Eutrophication leads to enhanced primary productivity of plant communities in grassland ecosystems. This intensifies the competition for light, allowing fast growing or taller species to outcompete other species. This competitive exclusion leads to dominance of a few plant species and results in the loss of biodiversity. Another impact of eutrophication on grassland communities is that it changes the ratio between major nutrients, such as N and P. Along the N:P gradient there is a shift in species composition, this implies the risk that eutrophication of a single element would change species composition and has a directional effect on the community structure (Fujita, 2010).

Plant traits concerning nutrient acquisition, retention and use possibly explain the ability of species to successfully compete for resources. Phenotypic plasticity allows organisms to adjust to a large range of conditions without evolutionary change. It is defined by Whitman and Agrawal (2009) as, '*the capacity of a single genotype to exhibit a range of phenotypes in response to variation in the environment*'. Genetic variation and phenotypic plasticity allow species' trait values to vary among sites in response to environmental filters and interactions with neighbouring species (Siefert, 2012). In general N-limitation reduces leaf area development and increases biomass allocation to the roots, whereas P-limitation generally stimulates biomass turnover, nutrient resorption for senescing tissue and root exudates production (Olde Venterink and Güsewell, 2010).

Phenotypic plasticity is beneficial when it allows an individual to alter its phenotype to adaptively match a changing environment (DeWitt and Langerhans, 2004). Species with a large phenotypic plasticity for nutrient acquisition and use are better able to cope with changes in nutrient availabilities than less plastic species (Berg and Ellers, 2010). These opportunistic species are expected to have a competitive advantage over specialistic species (which are less plastic) under eutrophication. Trade-offs between plant traits are a fundamental mechanism explaining the outcome of plant competition along nutrient gradients and changes in the amount of and/or balance between major nutrients caused by eutrophication. A species with better adaptive traits or more efficient plastic responses to a particular type of nutrient limitation is likely to be a competitive winner (Fujita, 2010).

Douma (2011) defines plant traits as "*morphological, physiological and phenological characteristics of plants, measurable at the individual level, from the cell to the whole organism level*". Under competition, effective pre-emption of soil nutrients, biomass allocation to roots as well as morphological and physiological properties of the roots are important for a rapid uptake of nutrients, which is critical for plant species performance (Olde Venterink and Güsewell, 2010). Grime (2002) defines competition as "*the tendency of neighbouring plants to utilise the same quantum of light, ion of mineral nutrient, molecule of water or volume of space*".

This research focused on the relation between plant traits and the N:P supply ratios as well as nutrient supply levels. The selected plant traits are specific leaf area (SLA; $\text{mm}^2 \text{mg}^{-1}$), canopy height (CH; m) and specific root length (SRL; $\text{cm g}^{-1} \text{root}$) (Douma, 2011; Ansquer, 2008; Ordóñez, 2009; Fujita, 2010a).

Specific Leaf Area

Traits describing the leaf economy strategy have been shown to depend strongly on water and nutrient supply (Ordonez et al., 2009).

Specific Leaf Area (SLA) is defined as *'The one-sided area of a fresh leaf divided by its oven-dry mass, hence leaf mass is one component of the SLA measurements, expressed as leaf dry mass, expressed in mm² mg⁻¹* (Kunzmann, 2005). It is a central component of the leaf economy strategy, which captures the trade-off between rapid resource acquisition and resource conservation (Siefert, 2012; Ordonez et al., 2009).

SLA is an important factor in determining the competitive ability of species along soil fertility gradients (Knops and Reinhart, 2000). Light perception is dependent on leaf area (Aerts et al., 1991) and SLA is positively correlated with nitrogen level (Aerts and Chapin, 2000; Knops and Reinhart, 2000). The SLA in canopies of fast growing perennials of productive habitats is often found to be high (Grime, 2002). These fast growing species are considered to be limited by the availability of N rather than P. Theoretically it is thus expected that there is a difference for SLA between N- and P-limited species, and that N-limited species have a higher SLA (Ansquer, 2008).

Canopy height

Canopy height is defined as *'The distance (m) between the highest photosynthetic tissue and the base of the plant'* (Kunzmann and Knevel, 2005). Plant height is a leading dimension of ecological variation, is important for the competitive ability and survival of plant species, and is shown to be related to many important functional traits (Westoby, 1998; Westoby et al., 2002; Poorter et al., 2003; Sun & Frehlich, 2011).

Canopy Height is at the core of the light competition strategy (Ordonez et al., 2009; Grime, 2002), where being taller than neighbours leads to having a competitive advantage through prior access to light. Increasing canopy height comes at the cost of construction and maintenance of non-productive tissues (Givnish, 1995) and provides investments in conductive tissues to maintain water transport (Mencuccini, 2003). A high nutrient supply level is expected to maximize the competition for light and thus to increase the canopy height (Ordonez et al., 2009). Soil N has a positive effect on max height. From this you theoretically expect a negative correlation with N:P ratio, meaning that when the N:P ratio is high (i.e. P-limitation) canopy height is expected to be lower than for a low N:P ratio (i.e. N-limitation).

Specific root length

Specific root length (SRL) is the ratio of root length to root mass (cm g⁻¹ root FW) (Fujita, 2010a) and describes the amount of absorptive tissue per unit mass of plant roots. Plants with a high SRL tend to build longer roots with a smaller diameter than plants with a low SRL. It is an important trait at the within-organ level.

Root architecture is an important determinant of the efficiency of exploration and exploitation of mobile soil resources. For mobile ions, such as nitrate, uptake is limited not by diffusion in the soil but by uptake at the root surface. Immobile ions such as phosphate are limited more by diffusion to the root surface (Hodge, 2004).

Plants may have a high nutrient uptake capacity as a result of a high biomass allocation to the roots, or they may have high uptake capacity by having a lower root allocation but a higher SRL. Nutrient uptake is dependent on the root length (Aerts et al., 1991) and is often better correlated with root length than with root mass (Fitter, 1991, in Aerts and Chapin, 2000).

Root length and root demography respond strongly to supply levels of nutrients. Fujita (2010a) showed that SRL decreased with increasing supply levels. Plants with a high N:P ratio allocate less biomass to roots than plants with the same growth rate but a low N:P ratio. Also the identity of neighbours can have a large impact on root morphological responses (Hodge, 2004).

This research will focus on the three selected plant traits for nutrient acquisition and use for six plant species with different growth strategies to test whether the competitive advantage of some species can be explained by trait plasticity under different N:P ratios and nutrient supply levels. The aim of this research is to examine the relative competitive ability of N- and P specialists and opportunistic plant species along a N:P stoichiometric gradient and to test whether this can be explained by trait plasticity of measured plant traits related to nutrient acquisition and use. The relative competitive ability (i.e. relative competitive strength or success ratio) is the ratio of the biomass produced in mixture divided by the biomass the same species produced in monoculture. By calculating the biomass ratio in mixture pots the relative dominance of the two species in competition can be assessed (Olde Venterink and Güsewell, 2010).

The following research question will be addressed. *Is there a difference in the relative competitive ability of N and P specialists and opportunistic plant species along a N:P stoichiometric gradient and can plant trait plasticity of traits related to nutrient acquisition and use explain the competitive advantage of certain species?*

This question can be divided in the following sub questions:

1. Is there a significant difference in biomass ratio (i.e. relative competitive strength), for different species combinations, supply levels, and N:P ratios?
2. Is there a significant difference in plant trait values between plant species, N and P supply levels and N:P ratios in monoculture and mixture?
3. Is there a significant difference in plant trait values within plant species for different N and P supply levels and N:P ratios in monoculture and mixture?
4. Is there a relation between the relative competitive strength and the plant trait values?

It is expected that N and P specialists produce significantly more biomass than opportunistic species under extreme limitation of nitrogen or phosphorus. Opportunistic species are expected to have a greater overall performance because they can adapt to a large range of nutrient limitation and supply levels due to a larger plant trait plasticity.

Species with a specialistic growth strategy are adapted to either N or P deficiency. However, being a specialist is expected to come at the expense of being less plastic with regard to the trait (i.e. specialistic species are expected to have a lower phenotypic plasticity). Opportunistic species are able to profit from N and P enrichment and therefore are more likely to have a competitive advantage under a larger range of N:P ratios and supply levels. Opportunistic species can profit from N and P enrichment because of their larger trait plasticity.

In more detail the following hypothesis per sub question were made:

1. Is there a significant difference in biomass ratio (i.e. relative competitive strength), for different species combinations, supply levels and N:P ratios?

- a. Due to the larger adaptability of opportunistic plant species it is expected that for these species the biomass ratio is larger than for specialistic species.
- b. At extreme N or P limitation N or P specialists are expected to have an overall higher success ratio compared to opportunistic species.
- c. Biomass production is expected to increase with increasing supply level.

2. Is there a significant difference in plant trait values between plant species, N and P supply levels and N:P ratios in monoculture and mixture?

- a. There is expected to be a significant difference in plant trait values between species. A significant difference in plant trait values between plant species, indicates interspecific differences.
- b. N specialists are expected to have a higher SLA compared to P-specialists.
- c. Canopy height and specific root length are expected to be significantly different between plant species.
- d. CH, SRL and SLA are expected to be negatively correlated with N:P ratio.
- e. CH and SLA are expected to be positively correlated with supply level.
- f. SRL is expected to decrease with increasing supply level.

3. Is there a significant difference in plant trait values within plant species for different N and P supply levels and N:P ratios in monoculture and mixture?

- a. Plant trait values of opportunistic species are expected to change with changing N:P stoichiometry. While for N and P specialistic species no significant difference in plant trait values is expected. Comparing the same species for different N and P supply levels and N:P ratios indicates intraspecific differences and thus shows the phenotypic plasticity.

4. Is there a relation between the relative competitive strength and the plant trait values?

- a. A relation between plant trait values and success ratio is expected, because these traits are expected to be critical for plant species performance

2.0 Materials and methods

In a greenhouse experiment the relationship between N:P supply ratio, nutrient supply level, trait plasticity and competitiveness was tested. The goal of this competition experiment was to provide insight in the impact of N:P stoichiometry, nutrient supply level and trait plasticity on plant competition.

Species were selected via a database analysis in which they were divided in N-limitation specialists, P-limitation specialists and opportunists.

2.0.1 Sowing

The seeds of 29 selected plant species were stored cold for about a week. All 29 plant species were sown on sowing- and propagating soil.

For the germination and first growth period a uniform temperature of 20 °C was chosen. After 12 days the selected species were placed in a colder greenhouse with a day temperature of 14 degrees and a night temperature of 12 degrees. The shift to a colder greenhouse was to limit growth of the already large enough individuals in order to reach an approximate similarity in biomass of the individuals used for the growth experiment.

After 17 days plant species for the growth experiment were selected (i.e. two N-specialists, two P-specialists and two opportunistic species). This selection was based on having a large enough (estimated) amount of individuals. The selected species are *Alopecurus pratensis* (N1), *Rumex acetosa* (N2), *Briza media* (P1), *Centaurea jacea* (P2), *Knautia arvensis* (O1), and *Prunella vulgaris* (O2).

2.0.2 Growth experiment

The growth experiment was conducted for six plant species, in monoculture and in mixture, over a time period of 9 weeks. During the experiment a uniform temperature of 18 °C was chosen.

The selected species were placed with four individuals in a pot, filled with 800 ml of quartz sand (Dorsilit, nr. 8, 0,3-0,8 mm). The soil material in the pots contained no nutrients or only negligible amounts. The average weight of a pot including sand, without plants, is 1,267 g.

The selected plant species were placed in monoculture and in mixture following a replacement design (Olde Venterink and Güsewell, 2010). The monoculture pots contained four seedlings of one species, while the mixture pots also contained four seedlings but two seedlings per species; the two species were arranged diagonally. In the competition experiment a N- or P-limitation specialist was combined with an opportunistic species (as indicated in table 1).

Table 1: Competition experiment, here the N- and P-specialists will be placed in mixture with the opportunistic species.

N1	O1
N1	O2
N2	O1
N2	O2
P1	O1
P1	O2
P2	O1
P2	O2

2.0.3 Nutrients

In this experiment different amounts of N and P were supplied for each treatment while all other nutrients were supplied in constant non-limiting amounts (totals per pot: 96 mg K, 206 mg Ca, 71 mg S, 47 mg Mg, 16 mg Fe, 1,55 mg Bo, 0,83 mg Mn, 0,4 mg Zn, 0,16 mg Mo, 0,08 mg Cu). In the experiment three supply levels of nutrients (high, medium and low) at five ratios of N:P (1.7, 5, 15, 45, 135) were combined, resulting in 15 different treatments (see table 2). Each treatment was replicated 5 times.

A modified Hoagland solution was used, this solution provides every nutrient necessary for plant growth and is appropriate for the growth of a large variety of species. The supply of N and P was determined such that their geometric means came out equal for all treatments at a distinct supply level, because it was expected that the effects of N and P supply were multiplicative; this is in line with previous studies (Güsewell, 2005, Fujita, 2010). The final nutrient solution was mixed just before application.

Over time the overall amount of nutrient solution per pot increased (to a maximum of 22,5 ml) in order to compensate for plant growth.

Table 2: The three supply levels of nutrients (high, medium and low) combined with five ratios of N:P (1.7, 5, 15, 45, 135). Resulting in 15 different treatments, with for each treatment the total amount of N and P (mg).

Supply level	N:P ratio	N	P
low	1,67	6,45	3,87
	5	11,18	2,24
	15	19,36	1,29
	45	33,54	0,75
	135	58,09	0,43
intermediate	1,67	19,36	11,62
	5	33,54	6,71
	15	58,09	3,87
	45	100,62	2,24
	135	174,28	1,29
High	1,67	58,09	34,86
	5	100,62	20,12
	15	174,28	11,62
	45	301,87	6,71
	135	522,85	3,87

2.0.4 Randomization

In order to limit the effects of spatial variation in the greenhouse all pots were rotated. Pots in the greenhouse were arranged in 5 blocks (see figure 2), each containing 420 pots, with one replicate per treatment. The pots were moved from one block to the next (1→2→3→4→5) at 1/5, 2/5, 3/5 and 4/5 of the duration of the experiment. Pot were randomly selected and placed in the next block.

Because block 1 and 2 have a different shape than the other three blocks we sub rotated the pots within these blocks. With the sub rotation one third of the pots was moved from the front to the back (A→B→C) of the greenhouse. This was done on every fourth and eighth day between the full rotations, the position within the sub block was not changed with this rotation.

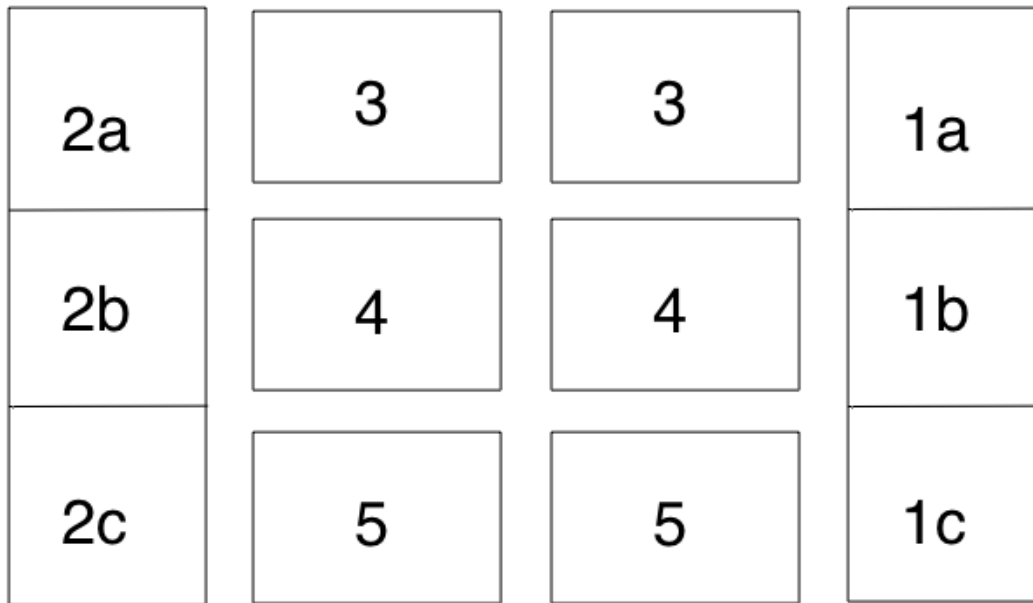


Figure 2: In the greenhouse pots were arranged in 5 blocks, each containing 420 pots, with one replicate per treatment.

2.1 Measurements

2.1.1 Final harvest

After 9 weeks of fertilization, measurements were performed for the selected vegetative traits and biomass. Due to differences in species (grasses and herbs) the length of the longest leaf is used instead of the canopy height. The length of the longest leaf still represents the ability of a species to compete for light.

For each pot the length of the longest leaf was determined, after which the four plant species were removed from the pot and carefully separated in water. For each individual root and shoot were separated.

The leaf fresh weight (in gram) and the leaf area of fresh leaves (in mm²; i.e. using a LI-COR LI-3100C Area Meter) were determined.

The roots were weighed to determine the root fresh weight (in gram) after which a sample of approximately 100 mg of root was taken. In order to determine the root length a scan of the 100 mg root was made. After these measurements the root and shoot for each individual were oven-dried (for 72 hours at 60 °C) and then weighed. Roots that grew out of the pot were collected separately because it wasn't possible to determine which part belonged to which individual. This 'root rest' was collected and then dried and weighed.

SLA was measured by determining the leaf area of fresh leaves, after which the leaves are dried and weighed. SLA is then calculated as the leaf area divided by the dry weight (mm² mg⁻¹).

SRL was calculated by dividing the root length by the root dry weight. The root length was determined via analysis of the digital images using Matlab. The root length was estimated from line intercepts, using the line intersect method of Tennant (1975). The intercepts of the roots with the vertical and horizontal grid line were counted, the longer the root, the more intercepts it makes. Counts were converted to length using the following formula: $R = \left(\frac{11}{28}\right) * N$, where R is the root length and N is the total number of intersections. Grid square size was 236 pixels for 0,5 cm width/length (see appendix 2 for the whole script).

2.1.2 Relative dominance

The success ratio was determined by calculating ratios of the biomass produced in mixture by that of the same species in monoculture. The five replicates of the mixture were compared with the mean biomass in monoculture. Monoculture biomass was multiplied by a factor of 0,5 to account for the presence of two versus four individuals per pot in mixture and monoculture.

A species is considered to be the superior competitor if it produces significantly more biomass in mixture than in monoculture (success ratio > 1) and/or causes the other species to produce significantly less biomass in mixture than in monoculture (success ratio < 1). By calculating the biomass ratio in mixture pots the relative dominance of the two species in competition can be assessed (Olde Venterink and Güsewell, 2010).

2.2 Statistical analysis

Because the variation within groups of variables was not normally distributed all data had to be transformed. The data for leaf length and SRL were square root transformed. The SLA, biomass (i.e. total biomass, leaf fresh weight, leaf dry weight, root fresh weight and root dry weight) and success ratio were log-transformed. After transformation the SRL, SLA and leaf fresh weight still weren't normally distributed, this increases the risk of a type 1 error. But when group sizes are equal the F-statistic can be quite robust to violations of normality (Field, 2009). For all variables there is no homogeneity of variance (Levene's test). Due to the large dataset used for this analysis, violations of homogeneity of variance are likely to occur.

Besides being a possibly robust test the QQ-plots of the trait values were acceptable, also a non-parametric test could not reveal any interaction effects which are needed to answer the research questions. Therefore despite of the increased risk of type 1 and 2 errors an ANOVA was used in the statistical analysis of the data.

2.2.1 Success ratio; Three-way independent ANOVA

With a three-way ANOVA, with Tukey post hoc test, the main effects of each individual independent variable (i.e. 'species', 'supply level' and 'N:P ratio') on the success ratio of a plant species were analyzed. Besides the main effects, the analysis also shows the interaction effects. Interaction effects show how much of the variance is explained by the interaction between the variables. In this analysis the dependent variable is the success ratio and the three independent variables are 'N:P ratio' (i.e. 1,7, 5, 15, 45, 135), 'species' and 'supply level' (i.e. low, medium or high).

2.2.2 Plant trait values; three-way independent ANOVA

In order to determine the effects of N:P supply ratio, N and P supply levels and species on various plant trait values (i.e. leaf length, SLA and SRL) a three-way independent ANOVA with Tukey post hoc test was used. Besides these main effects the interaction effects will also be included.

2.2.3 Phenotypic plasticity; ANOVA

Phenotypic plasticity represents a measurable variation and thus can be measured by ANOVA with a Tukey post hoc test. The data file was split by species, after which a three-way independent ANOVA was run for the leaf length, SLA and SRL to test whether or not there was phenotypic plasticity in traits.

2.2.4 Correlation; multiple linear regression

In order to determine the relation between plant trait values and the relative competitive strength of a species, a multiple linear regression (forced entry) was used. This regression tests whether there is a relationship between the success ratio and the plant trait values of a species.

The linear regression showed which amount of the variation in the success ratio can be explained by plant trait values.

3.0 Results

No significant differences between monocultures and mixtures were found for the total biomass ($F(1,1638)=2,3$, n.s.), SLA ($F(1,1433)=0,76$, n.s.), and SRL ($F(1,654)=0,004$, n.s.; one-way ANOVA). Only for leaf length there is a significant difference between an individual in monoculture or competition ($F(1,1449)=6,6$, $p<0,05$), in monoculture individuals have a significantly higher leaf length than in mixture.

The block effect was tested with a one-way ANOVA split in monocultures and mixtures for all dependent variables. In monoculture there was no significant block effect for leaf length ($F(1,651)=2,4$, n.s.) and total biomass ($F(1,709)=0,33$, n.s.). For SLA ($F(1,641)=16,0$, $p<0,001$) and SRL ($F(1,319)=9,2$, $p<0,01$) there was a significant block effect in monoculture, the SLA is significantly lower in block 2 while SRL in block 2 is significantly higher. In mixture there is no significant block effect for leaf length ($F(1,797)=0,05$, n.s.) and SRL ($F(1,334)=2,99$, n.s.). For the other variables there is a significant block effect, total biomass ($F(1,928)=7,1$, $p<0,01$) is shown to be higher in block 2, SLA ($F(1,791)=28,8$, $p<0,001$) is significantly lower in block 2, and the success ratio ($F(1,917)=5,4$, $p<0,05$) in block 2 is significantly higher.

3.1 Interspecific differences

Overall the main effects of species, supply level and supply ratio are significant for all variables (see table 3). In the following section the results are described per variable.

Table 3: Effects of species (SP), nutrient supply levels (L), and N:P supply ratios (NP) and their interactions on the total biomass, success ratio, leaf length, SLA and SRL. F values and P values (** $p<0,001$; * $p<0,01$; * $p<0,05$) were determined by a three-way independent ANOVA.

ANOVA results	df	Total biomass (g)	Success ratio	Leaf length (cm)	SLA (mm ² g ⁻¹)	SRL (cm g ⁻¹)
Species	5	153,78***	270,73***	1676,78***	12,37***	17,89***
Level	2	583,10***	1191,46***	847,91***	7,09**	32,51***
N:P ratio	4	122,45***	245,92***	125,71***	1,93 n.s.	8,996***
Level*SP	10	3,69***	5,94***	2,22*	1,496 n.s.	0,92 n.s.
NP*SP	20	4,25***	7,63***	4,00***	1,74*	2,697***
NP*level	8	5,27***	9,34***	6,59***	2,73**	1,56 n.s.
NP*L*SP	40	1,78***	3,42***	2,96***	1,69**	1,32 n.s.
error		1548	828	1360	1343	564

3.1.1 Effects on total biomass

Average total biomass was significantly influenced by nutrient supply level ($F(5,1548)=153,8$, $p<0,001$) and N:P ratio ($F(4,1548)=122,4$, $p<0,001$) (see table

3). The overall mean biomass was highest in pots receiving the highest nutrient supply (see figure 3). For N:P ratio the Tukey post hoc test shows that a ratio of 135 and 45 lead to a significantly lower total biomass. Total biomass was significantly lower for a ratio of 15 than for 5 (see figure 4).

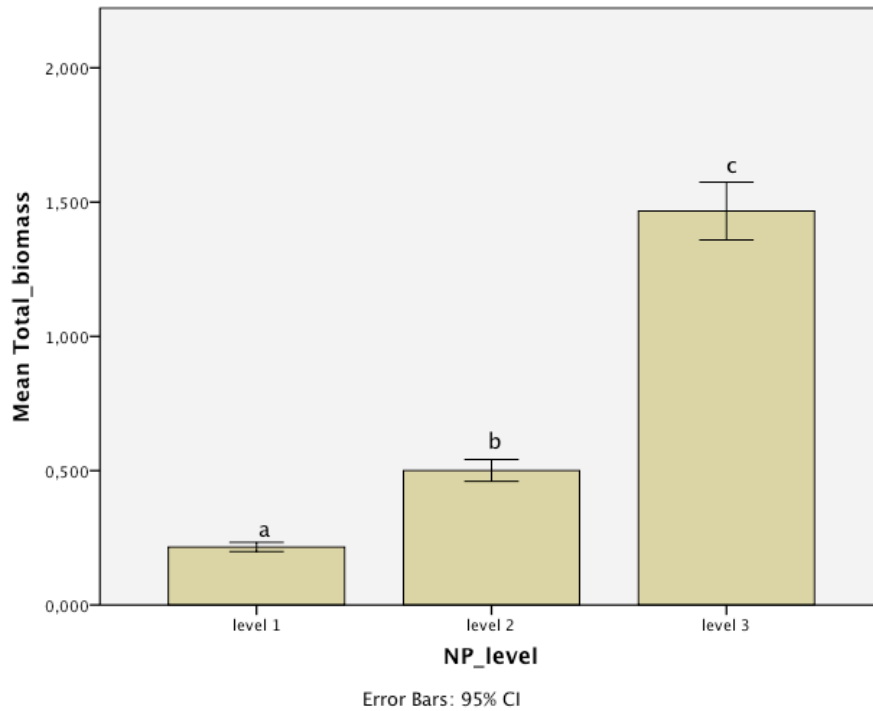


Figure 3: The mean total biomass per nutrient supply level. The outcome of the three-way independent ANOVA showed there is a significant main effect of supply level on the total biomass ($F(5,1548)=153,8, p<0,001$). The biomass increases with the nutrient supply level.

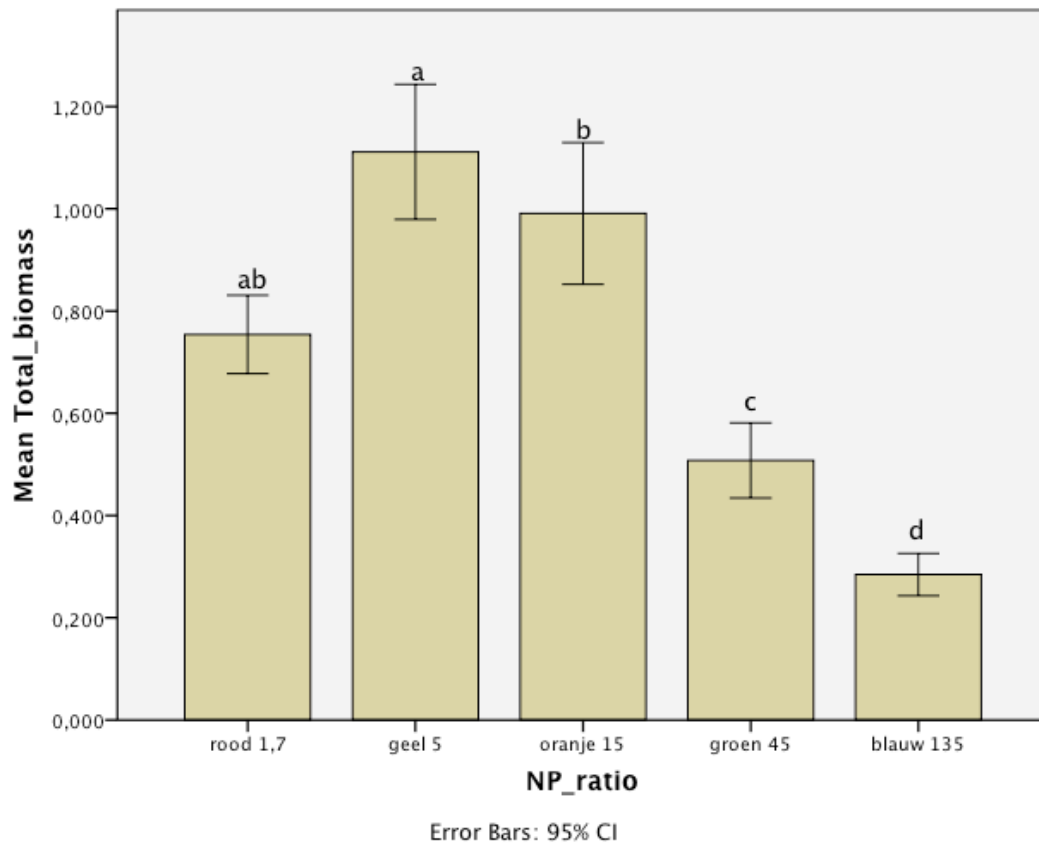


Figure 4: The mean total biomass per N:P supply ratio ($F(4,1548)=122,4, p<0,001$). The total biomass is higher at low to intermediate N:P supply ratios.

There was a significant main effect of species ($F(5,1548)=153,8, p<0,001$) on the total biomass. P-specialists, N-specialists and opportunists were not significantly different from each other, but between P, N and O there was a significant difference in total biomass ($N1=N2 > P1=P2 > O1=O2$). The N-specialists had the highest total biomass followed by the P-specialists, opportunistic species had the lowest total biomass.

There was a significant interaction effect between the N:P ratio and nutrient level ($F(8,1548)=5,3, p<0,001$), indicating that the effect of the nutrient level on total biomass differs per N:P ratio. The difference in total biomass is larger under N-limitation than under P-limitation.

There was a significant interaction effect between the N:P ratio and species on the total biomass ($F(20,1548)=4,2, p<0,001$). Meaning that plant species respond differently to N:P ratios (figure 5). An ANOVA performed on the data split by N:P ratio showed that under N-limited conditions (i.e. a N:P ratio of 1,7 or 5) specialistic species perform significantly ($p<0,001$) better than opportunistic species. Under P-limitation the N specialists (*Alopecurus pratensis* and *Rumex acetosa*) performed significantly better than P-specialists or opportunists.

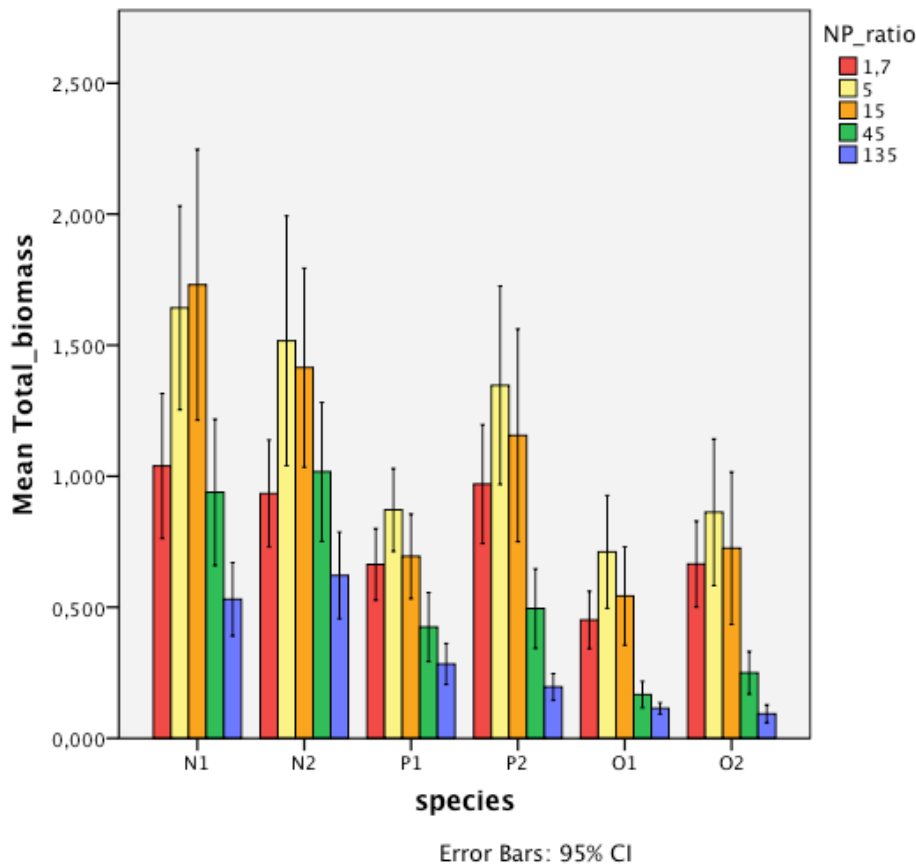


Figure 5: For total biomass the interaction effect between species and N:P ratio is significant (three-way ANOVA; $F(20,1548)=4,2, p<0,001$), which indicates that between species the difference between the N:P ratios is different. Under N-limited conditions the mean total biomass of specialists is significantly ($p<0,001$) higher than for opportunists, under P-limited conditions the N specialists still have the significantly highest total biomass.

There was a significant interaction effect between the nutrient supply level and species on the total biomass ($F(10,1548)=3,7, p<0,001$), indicating that the difference in total biomass between nutrient levels varies per species.

The three way interaction shows, Level*ratio*species ($F(40,1548)=1,8, p<0,05$), that differences exist between nutrient levels in how the total biomass of plants is affected by the N:P ratios and species. At level 2 and 3 a N:P ratio of 5 leads to the overall highest total biomass for all species. For all supply levels and N:P ratios the total biomass of N-specialists is highest, followed by P-specialist and opportunistic species have the lowest total biomass. This pattern is more clear when the nutrient level is low.

3.1.2 Effects on success ratio

There was a significant main effect of species ($F(5,918)=270,7, p<0,001$) on the success ratio. In general opportunistic species have a lower success ratio than specialistic species. Success ratio was significantly highest for N-specialists, followed by P-specialists and lowest for the opportunistic species (figure 6; $N1=N2, P1=P2, O1=O2$).

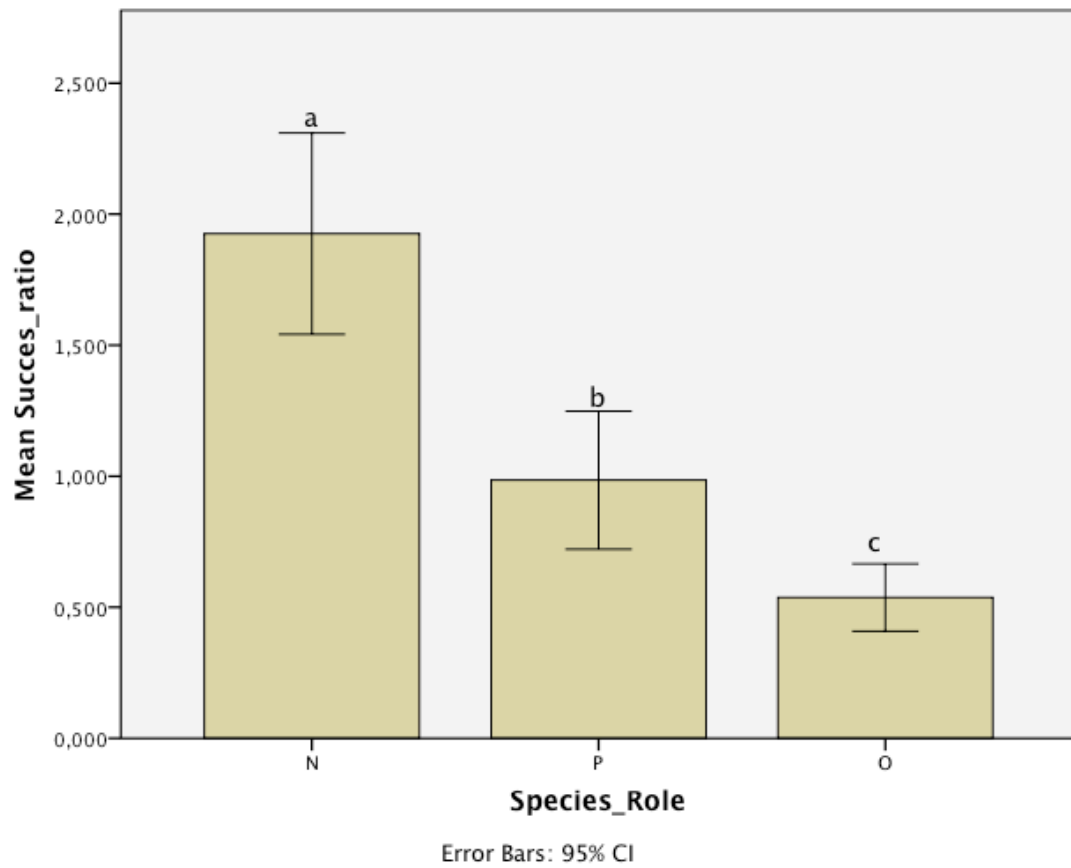


Figure 6: The mean success ratio per species role. The outcome of the three-way independent ANOVA indicates that there is a significant main effect of species on the success ratio ($F(5,918)=270,7, p<0,001$).

There was a significant main effect of N:P ratio ($F(4,918)=245,9$, $p<0,001$) on the success ratio. A N:P supply ratio of 135 leads to the lowest success ratio, total success ratio was highest for a ratio of 5 (Tukey post hoc test).

A significant main effect was also found for the nutrient level ($F(2,918)=1191,5$, $p<0,001$) on the success ratio. A positive relation between the nutrient supply level and success ratio was found (i.e. success ratio increases with supply level).

There was a significant interaction effect between the N:P ratio and N:P level on the success ratio ($F(8,918)=9,3$, $p<0,001$). A more detailed ANOVA (split by N:P ratio) indicated that for each N:P supply ratio the success ratio in level 3 is significantly higher than in level 2, which is significantly higher than in level 1 ($p<0,001$).

There was a significant interaction effect between the N:P ratio and species on the success ratio ($F(20,918)=7,6$, $p<0,001$). This indicates that the difference in success between N:P ratios is different per species (as shown in figure 7). An ANOVA split by N:P ratio shows that for each N:P ratio the success ratio is significantly different per species ($p<0,001$). The N-specialists have the significantly highest success ratio for all N:P supply ratios while for opportunists the success ratio is lowest (see appendix 3 for the post hoc tests).

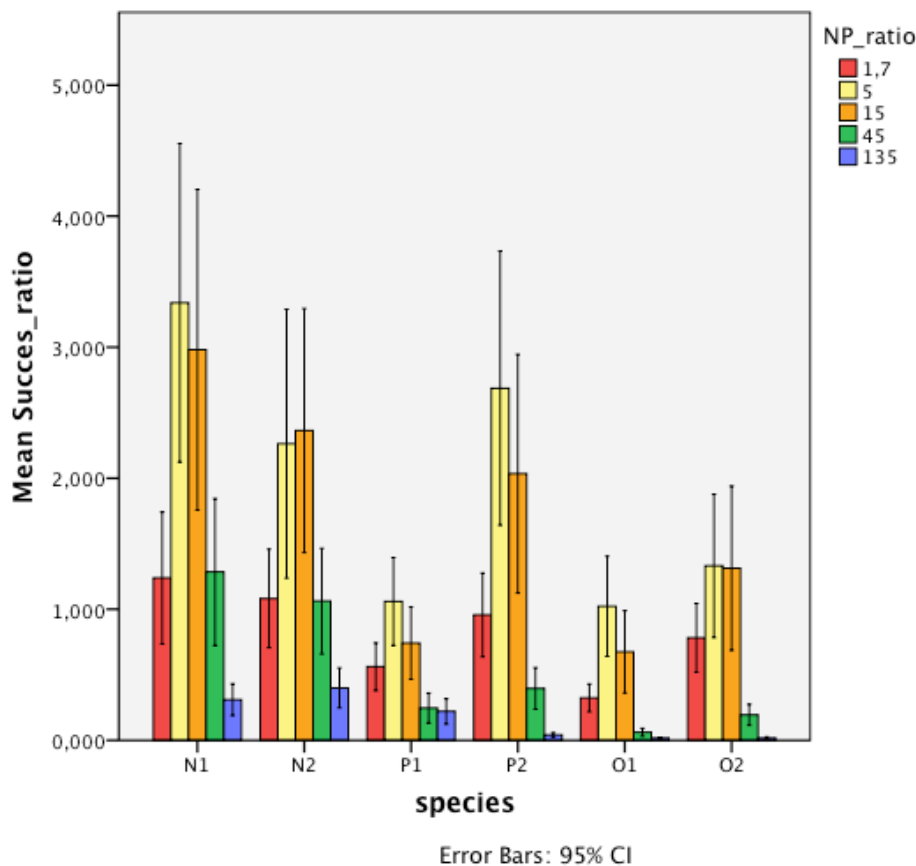


Figure 7: The interaction effect between species and N:P ratio, which is shown by ANOVA to be significant ($F(20,918)=7,6$, $p<0,001$). This indicates that between species the difference between the N:P ratios is different. An ANOVA per N:P ratio shows that for each N:P ratio the success ratio is significantly different per species ($p<0,001$).

There was a significant interaction effect between the nutrient level and species ($F(10,918)=5,9$, $p<0,001$), which indicates that the difference in success ratio between species is different per nutrient level. For each nutrient supply level the success ratio of the N-specialists is significantly higher than for opportunists, only in level 1 there is a significant difference per functional group ($0<P<N$; figure 8).

NP_level=level 1

Tukey HSD^{a,b}

species	N	Subset for alpha = 0.05		
		1	2	3
O2	63	-5,1437		
O1	76	-4,6820		
P2	39		-3,6643	
P1	44		-3,3300	
N2	40			-2,2336
N1	36			-2,2084
Sig.		,326	,682	1,000

Means for groups in homogeneous subsets are displayed.

- a. Uses Harmonic Mean Sample Size = 46,091.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

NP_level=level 2

Tukey HSD^{a,b}

species	N	Subset for alpha = 0.05			
		1	2	3	4
O1	73	-3,6786			
O2	76	-3,3325			
P2	36		-2,0265		
P1	36		-1,6363	-1,6363	
N1	44			-,9985	-,9985
N2	40				-,6619
Sig.		,890	,829	,352	,901

Means for groups in homogeneous subsets are displayed.

- a. Uses Harmonic Mean Sample Size = 46,104.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

NP_level=level 3

Tukey HSD^{a,b}

species	N	Subset for alpha = 0.05		
		1	2	3
O1	80	-1,3125		
O2	72	-,5034	-,5034	
P1	37		,0242	
P2	46		,3181	,3181
N2	36			1,1992
N1	44			1,2476
Sig.		,162	,149	,069

Means for groups in homogeneous subsets are displayed.

- a. Uses Harmonic Mean Sample Size = 47,748.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Figure 8: The Tukey post hoc test of a One-Way ANOVA split by nutrient supply level ($p<0,001$) shows that the success ratio of N-specialists is significantly higher than that of opportunists for all N:P supply levels.

The three way species*NPllevel*NPratio interaction is significant ($F(40,918)=3,4, p<0,001$) meaning that per nutrient level the effect of N:P ratio and species on the success ratio of an individual is different.

3.1.3 Effects on leaf length

For the leaf length the main effect of N:P ratio ($F(4,1360) = 125,7, p<0,001$), nutrient level ($F(2,1360) = 847,9, p<0,001$) and species ($F(5,1360) = 1676,8, p<0,001$) were significant. Leaf length increased significantly with supply level (Tukey post hoc test).

The leaf length was significantly different between N:P ratios, except for 1,7 and 15.

Between plant species the leaf length was significantly different for each species, the leaf length of both N and P specialists was significantly different. *Alopecurus pratensis* (N1) had the highest leaf length and the leaf length of the opportunistic species (O1 and O2) was significantly lower than for all other species. The leaf length of the opportunistic species was not significantly different from each other (see figure 9).

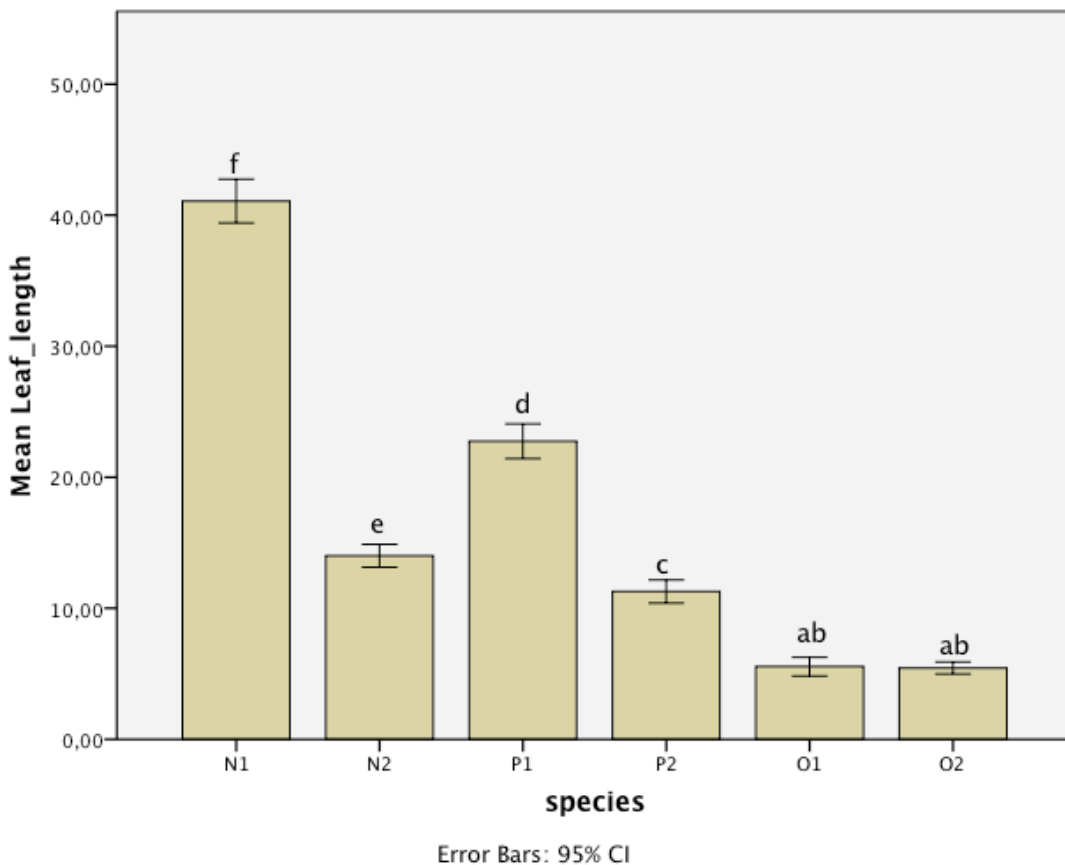


Figure 9: The leaf length (cm) per species. Between all species there is a significant difference in leaf length ($F(5,1360)=1676,8,p<0,001$), except between both opportunistic species (O1 and O2).

Interactions between N:P ratio and nutrient level ($F(8,1360)=6,6, p<0,001$) and N:P ratio and species ($F(20,1360)=4,0, p<0,001$) were significant. At extreme N- or P-limitation the leaf length is significantly highest in level 3 but does not differ between level 1 and 2 ($1=2<3; p<0,001$), for all other N:P supply ratios the leaf length is highest in level 3 and lowest in level 1 ($1<2<3; p<0,001$). The interaction effect between nutrient level and species was significant ($p<0,05$). For all nutrient supply levels the leaf length of both opportunistic species is significantly lowest ($p<0,001$; One-Way ANOVA split by level), *Alopecurus pratensis* has the highest leaf length ($O1=O2<P2<N2<P1<N1$).

The three way interaction is significant ($F(40,1360)=2,96, P<0,001$), indicating that depending on the nutrient level the effect of the N:P ratio and plant species on the leaf length of an individual is different.

3.1.4 Effects on SLA

The SLA was not influenced by the N:P ratio ($F(4,1343)=1,9$, n.s.).

The main effect of species ($F(5,1343)=12,4, p<0,001$) on the SLA was significant. The SLA of N2 and P2 was significantly higher than for the other species (Tukey post hoc test), but the specific leaf area of N2 and P2 did not significantly differ from each other.

Plants in level 2 had a higher SLA than those in level 3 ($F(2,1343)=7,1, p<0,05$), the SLA in level 1 was not significantly different from the other nutrient supply levels.

There is a significant interaction effect between N:P ratio and nutrient level ($F(8,1343)=2,73, p<0,05$) as well as between N:P supply ratio and species ($F(20,1343)=1,74, p<0,05$). The SLA between N:P supply levels is not significantly different for N:P supply ratios (One way ANOVA split by N:P ratio; n.s.), except for a N:P ratio of 135 where the SLA at level 2 is significantly higher than in level 1 ($1=3<2=3; p<0,05$).

The three way interaction is significant ($F(40,1343)=1,69, P<0,05$), indicating that depending on the nutrient level the effect of the N:P ratio and plant species on the SLA of an individual is different.

3.1.5 Effects on SRL

For the SRL the main effects of N:P ratio ($F(4,564)=9,0, p<0,001$), nutrient level ($F(2,564)=32,5, p<0,001$) and species ($F(5,564)=17,9, p<0,001$) were significant. The Tukey post hoc test showed that for N:P ratios of 45 and 135 the SRL was significantly higher than for low to intermediate N:P supply ratios (i.e. 1,7; 5; 15). The SRL was significantly different for all nutrient levels, in level 1 it was significantly higher than in level 3 and 2 ($1>2>3$). For plant species the SRL of N2 and P1 compared to the other species was significantly lower.

The effect of N:P ratio*species is the only significant interaction effect ($F(20,654)=2,7, p<0,001$). indicating that the difference in SRL between N:P ratios is different per species. For low to intermediate N:P supply ratios the SRL is not significantly different between species, only for a high N:P supply ratios there is a significant difference between species. For both N:P supply ratios the SRL of N2 is lowest, difference between these two ratios is that O1 is in the subset with the highest SRL for a N:P supply ratio of 45 but for a N:P supply ratio of 135 in the subset with the lowest SRL (see figure 10).

NP_ratio=45

Tukey HSD^{a,b}

species	N	Subset for alpha = 0.05		
		1	2	3
N2	21	39,6251		
P1	22	45,0069	45,0069	
N1	23		54,6549	54,6549
O1	22			59,2290
O2	25			59,5935
P2	20			60,4631
Sig.		,608	,056	,525

Means for groups in homogeneous subsets are displayed.

- a. Uses Harmonic Mean Sample Size = 22,058.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

NP_ratio=135

Tukey HSD^{a,b}

species	N	Subset for alpha = 0.05	
		1	2
N2	24	42,1737	
O1	21	51,4628	51,4628
P1	23	52,3754	52,3754
P2	25		58,7303
O2	20		58,8928
N1	21		59,2277
Sig.		,085	,323

Means for groups in homogeneous subsets are displayed.

- a. Uses Harmonic Mean Sample Size = 22,191.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Figure 10: The Tukey post hoc tests from a One-Way ANOVA split by N:P ratio. When the N:P ratio is high there is a significant difference in SRL between species. This outcome shows that the effect on SRL per N:P ratio is different. Difference between these two ratios is that O1 is in the subset with the highest SRL for a N:P supply ratio of 45 but for a N:P supply ratio of 135 in the subset with the lowest SRL

3.2 Intraspecific variation in plant traits

Intraspecific differences are found for all traits and all species, but not all species show intraspecific variation for each trait (table 4).

Table 4: Intraspecific variation in plant traits shown for N:P ratios and nutrient supply levels. Mean trait values per species and P values (**p<0,001; **P<0,01; *p<0,05) were determined by an ANOVA.

	error	Leaf length (cm)	SLA (mm ² g ⁻¹)	SRL (cm g ⁻¹)
N:P supply ratio	Df=4			
<i>Alopecurus pratensis</i>	206	6,28***	4,97 n.s.	54,79 n.s.
<i>Rumex acetosa</i>	198	3,63***	5,28 n.s.	43,03 n.s.
<i>Briza media</i>	195	4,66***	5,07*	47,57 n.s.
<i>Centaurea jacea</i>	203	3,12***	5,29 n.s.	51,50***
<i>Knautia arvensis</i>	233	1,92***	5,15*	52,32**
<i>Prunella vulgaris</i>	235	1,99***	5,21 n.s.	54,34**
Nutrient supply level	Df=2			
<i>Alopecurus pratensis</i>	206	6,28***	4,97 n.s.	54,79**
<i>Rumex acetosa</i>	198	3,63***	5,28**	43,03 n.s.
<i>Briza media</i>	195	4,66***	5,07 n.s.	47,57**
<i>Centaurea jacea</i>	203	3,12***	5,29 n.s.	51,50**
<i>Knautia arvensis</i>	233	1,92***	5,15 n.s.	52,32*
<i>Prunella vulgaris</i>	235	1,99***	5,21*	54,34***

3.2.1 Leaf length

Running the ANOVA for all individual species showed that the main effects of N:P ratios and nutrient level on leaf length were significant for all species (see table 2). This indicates that within plant species there is a significant difference in leaf length between nutrient supply levels and N:P ratios.

At extreme P limitation the leaf length for *Alopecurus pratensis* is significantly lower than the leaf length for other ratios (p<0,001; Tukey post hoc test). For *Rumex acetosa* the leaf length of N2 is highest for a N:P ratio of 5 and lowest extreme P-limitation (i.e. N:P ratio of 135). At intermediate to low N:P ratios the leaf length of *Briza media* is highest, while the lowest leaf length was found under P-limited conditions (i.e. a N:P ratio of 45 and 135). For an intermediate to low N:P ratio (15, 5, and 1,7) the leaf length of *Centaurea jacea* is significantly higher than for the high N:P ratios. At extreme P-limitation (i.e. N:P ratio of 135) the leaf length of P2 is lowest. *Knautia arvensis* has a significantly higher leaf length for intermediate to low N:P ratios (15, 5 and 1,7) compared to

high P-limited conditions (N:P ratio of 45 and 135). The leaf length of *Prunella vulgaris* was highest for a N:P ratio of 5, this was significantly higher than all other ratios but did not significantly differ from a N:P ratio of 1,7. The lowest leaf length was for a N:P ratio of 135 (see figure 11).

There is a positive effect of the nutrient supply level on the leaf length of all species.

For all species, except for the P-specialists, ANOVA show that the leaf length in competition is significantly different in monoculture than in mixture. The N-specialistic species, *Alopecurus pratensis* ($F(1,206)=8,7$, $p<0,01$) and *Rumex acetosa* ($F(1,198)=37,5$, $p<0,001$), have a significantly higher leaf length in mixture. For the opportunists, *Knautia arvensis* ($F(1,233)=16,4$, $p<0,001$) and *Prunella vulgaris* ($F(1,235)=28$, $p<0,001$), the leaf length in monoculture is significantly higher than in competition.

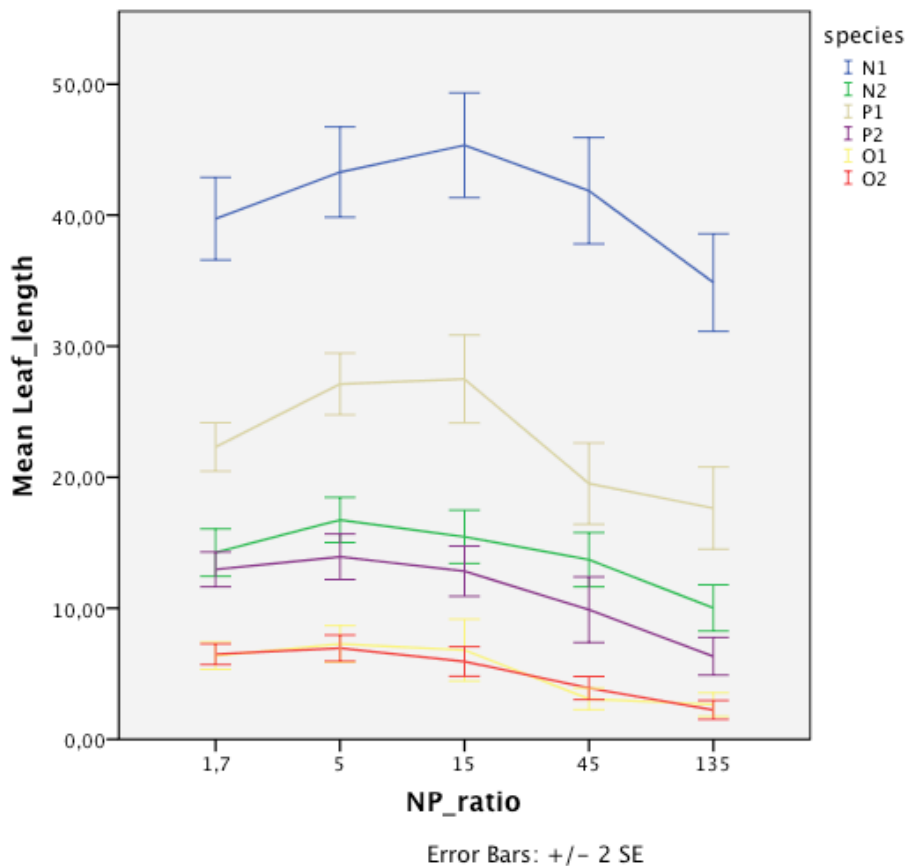


Figure 11: The mean leaf length per N:P ratio for each species. The outcome of the ANOVA shows that for all species the leaf length significantly differs per N:P supply ratio ($p<0,001$).

3.2.2 Specific Leaf Area

SLA between N:P supply ratios was only significant for *Briza media* ($p<0,001$) and *Knautia arvensis* ($p<0,001$). For *Briza media* the SLA at a N:P supply ratio of 15 is significantly higher than for a ratio of 45. For *Knautia arvensis* the SLA at an intermediate N:P supply ratio is significantly higher than at a ratio of 5.

Between nutrient supply levels the SLA was only significantly different for *Rumex acetosa* and *Prunella vulgaris*. The SLA of *Rumex acetosa* is significantly lower in level 3 compared to level 1 and 2, and for *Prunella vulgaris* the SLA is significantly higher in level 2 than in level 3.

3.2.3 Specific Root Length

The outcome of the ANOVA showed that there were significant main effects of nutrient supply levels and N:P ratios on the SRL. The nutrient supply level was significant for each species (except for *Rumex acetosa*) and showed the same general pattern of a significantly higher SRL when nutrient supply was low.

For *Alopecurus pratensis*, *Briza media*, and *Rumex acetosa* no significant main effects of N:P supply ratio on the SRL were found. For *Centaurea jacea* under P-limited conditions (N:P ratio of 45 and 135) the SRL was significantly higher than under intermediate or N-limited conditions. For intermediate to low N:P ratios the SRL of *Knautia arvensis* was significantly lower than for a N:P ratio of 45. The SRL of *Prunella vulgaris* was significantly lower for a N:P ratio of 5 and 15 than for a N:P ratio of 45 and 135.

3.3 Regression

For the regression analysis the log-transformed success ratio was used as the dependent variable, independent variables were the leaf length, SRL and SLA.

The outcome of the multiple regression shows that between leaf length and success ratio there is a positive relation ($r=0,578$, $p<0,001$). Between success ratio and SLA ($r=-0,211$, $p<0,001$) and SRL ($r=-0,353$, $p<0,001$) there is a negative relation.

When only leaf length is used as a predictor, 33,4% of the variability in success ratio is explained by the leaf length ($R^2=0,334$, $\beta=0,578$, $p<0,001$). Inclusion of SRL increases the amount of explained variation in success ratio to 41,0% ($R^2=0,410$, $\beta=-0,278$, $p<0,001$). Inclusion of SLA causes a further increase in the amount of explained variation in success ratio to 42,5% ($R^2=0,425$, $\beta=-0,125$, $p<0,01$).

Overall the outcome of the regression shows that the included plant trait values, which are leaf length and SRL, explain 42,5% of the variation in the success ratio.

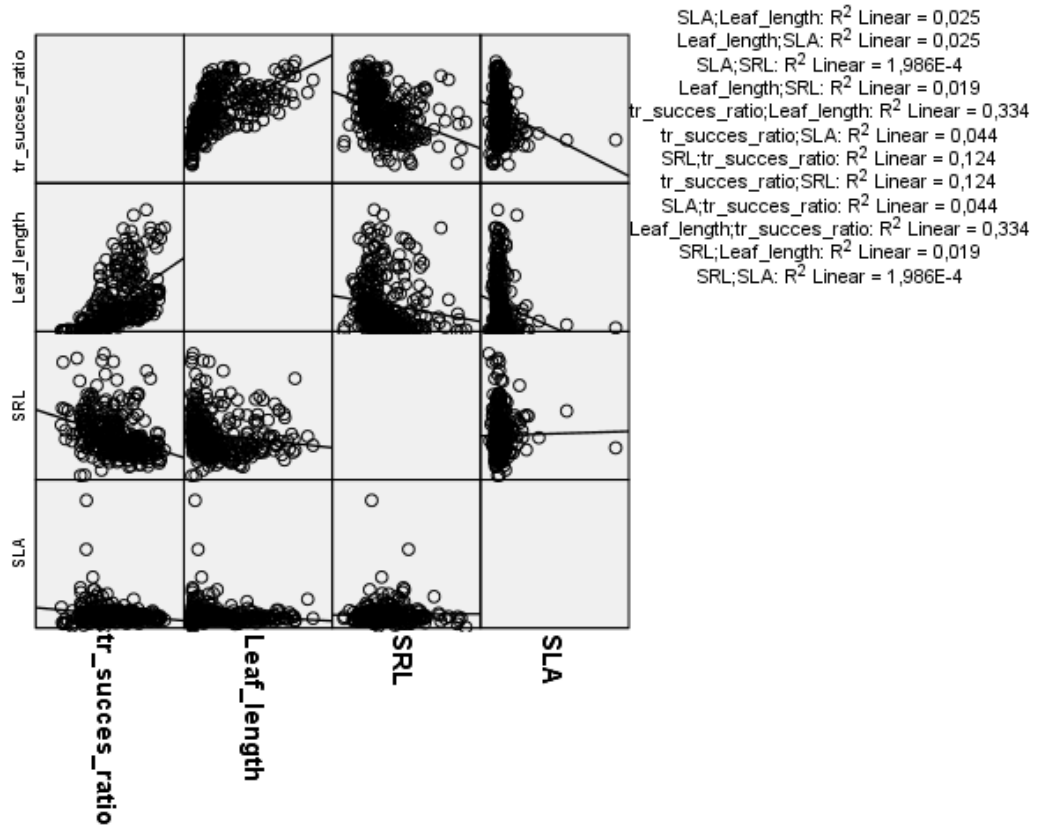


Figure 12: Scatter plots for the leaf length, SRL, SLA and the (log transformed) success ratio.

4.0 Discussion

The present study shows that plant growth and competitive ability are affected by the availability of N and P in terms of N:P supply ratio and nutrient supply level.

4.1 Interspecific differences

The outcome indicates a positive relation between biomass production and nutrient supply level. The intermediate N:P supply ratio (15) and the slightly N-limited treatment (5) have the highest total biomass. Under extreme nutrient limitation all species produce significantly less biomass, performing significantly better under N-limitation than under P-limited conditions. This result is comparable to those of previous similar experiments (Fujita et al., 2010b; Güsewell and Bollens, 2003). The lower biomass production under P-limitation than under N-limitation might indicate that the productivity curve under P-limitation is narrower than under N-limitation, which matches the findings of Wassen et al. (2005). This supports the theory of Olde Venterink et al. (2003), who state that a shift from one limiting nutrient to the other could have severe effects on the local species richness.

The biomass production was also influenced by species, even more by species group. The N-specialists had the highest total biomass followed by the P-specialists, opportunistic species had the lowest total biomass. This does not match the hypothesis, as was expected that opportunistic species would have a higher overall biomass production due to their larger adaptability. The analysis shows that overall the N-specialists perform best and that under extreme nutrient limitation all species produce significantly less biomass. As expected N and P specialists have a higher produced biomass under extreme nutrient limitation compared to opportunistic species. But the outcome of the ANOVA, split by N:P ratio, shows that N-specialists had the highest total biomass, even under extremely P-limited conditions. The short-term responses could be very different from the long-term responses (Güsewell, 2004; Fujita et al., 2010b). In such a short-term experiment differences in biomass production could only relate to either differences in nutrient uptake or to differences in nutrient productivity (Güsewell and Bollens, 2003). Nutrient storage and resorption are processes that become important in the long term and may lead to different results.

For the success ratio the results indicate that opportunistic species have the lowest success ratio and that the success ratio of N-specialists is the highest. High N:P ratios show the lowest success ratio while for intermediate and slightly N-limited conditions the success ratio is highest. With the success ratio under slightly N-limited conditions (i.e. a N:P ratio of 5) being significantly higher than under intermediate conditions. These findings do not match the hypothesis, as it was expected that the success ratio of opportunistic species would be highest.

For the leaf length the results indicate a significant difference between nutrient levels. Plants in level 3 have a larger leaf length indicating that a larger nutrient availability increases the competition for light; this is in line with the hypothesis. Under P-limitation the leaf length is significantly lower than under N-limitation, this is in line with the hypothesis as it was expected that soil N has a positive effect on plant height. Between species a significant difference in leaf length was found. Overall the leaf length of opportunists is significantly lower than for specialists. *Alopecurus pratensis* and *Briza media* (both grasses) have a significantly higher leaf length than all other species (all herbs).

The findings for the specific leaf area did not match the hypothesis. The analysis showed that SLA is influenced by the plant species and nutrient supply level. An effect was found between individual species but not between N and P specialists. The results show that the SLA in the intermediate supply level is higher than at the high nutrient supply level, while the SLA in productive habitats was expected to be higher. SLA was not significantly influenced by the N:P supply ratios. Güsewell (2005) also found non consistent patterns of N:P supply ratios on leaf morphology (SLA). She found that specific leaf area was only weakly related to N:P supply ratio, it mainly depended on plant species and only partly on nutrient supply. SLA was found to be more affected by light intensity than nutrient supply (Güsewell, 2005).

The significant negative correlation between N:P ratio and SRL is in line with the hypothesis and previous research of Fujita et al. (2010a). Fujita et al. (2010a) also showed a large variation in SRL among species, which matches with the outcome of this research. SRL was expected to decrease with increasing supply level, as is also seen in the outcome. The general pattern found for SRL is that under low nutrient supply (level 1) the SRL is higher than under high nutrient supply (level 3). The results show that SRL is an important trait for nutrient acquisition, interspecific differences in nutrient uptake might be due to differences in SRL.

Overall most interactions effects are significant, indicating the importance of looking at the effect of nutrient level as well as the N:P ratio on biomass and traits of different plant species. Interactions between N:P supply ratio and N:P supply level occurred for total biomass production, success ratio, leaf length and SLA. Indicating a different effect of N:P ratio per supply level. ANOVA showed species specific effect of N:P ratios on plant trait values.

4.2 Intraspecific differences

Plant trait values within species were shown to be significantly affected by the N:P supply ratio. Leaf length was shown to be significantly different between N:P supply ratios for each species, all species have a significantly lower leaf length for extreme P-limitation (i.e. a N:P ratio of 135), indicating phenotypic plasticity for this trait for all species. The outcome of this research shows no significant effect between N:P supply ratios on SLA for *Alopecurus pratensis* (N1), *Rumex acetosa* (N2) and *Prunella vulgaris* (O2), for all other species the effect was significant. Between N:P supply ratios the SRL was not significant for *Alopecurus pratensis* (N1), *Rumex acetosa* (N2), and *Briza media* (P1), for all other species the effect was significant.

This implies that not only opportunistic species show phenotypic plasticity in traits and that plasticity is different per trait per species. In general all species show plasticity in traits, but not for each trait. Only *Knautia arvensis* (O1) shows plasticity in all traits, but this does not match with the overall low performance of this species. Best performing species is *Alopecurus pratensis* (N1), this species has the highest success ratio, but for this species only for the leaf length phenotypic plasticity was shown.

These findings indicate that phenotypic plasticity in this selection of traits (i.e. leaf length, SLA and SRL) is not important for explaining the success ratio of a species. This study focused on the short term and it could be that plasticity effects that are found here might be different from the effects on the long term (Berg and Ellers, 2010).

4.3 Success ratio

The outcome of the regression analysis shows that only 42,5% of the data is explained by the leaf length, SLA and SRL, this result indicates that more than half of the variation in success ratio is explained by other factors.

Although much of the variation in success ratio is explained by other factors, the outcome does indicate a relation between the leaf length, SLA and SRL and the success ratio of a species.

Contrary to previous studies where SLA is often reported as being the most important factor (Knops and Reinhart, 2000), the SLA is the least important factor in determining the competitive ability of an species.

Plots show that assumptions of linearity and homoscedasticity might not have been met. The plot for leaf length is funnel shaped, which indicates that there is heteroscedasticity, this indicates increasing variance across the residuals. The plot for SLA indicates that the assumption of linearity might be violated. The histogram indicates a deviation from normality, this is not very large but could possibly pose problems with the analysis. Not meeting the assumptions means that the model probably could not generalize beyond this data.

4.4 Limitations of the research

4.4.1 Data transformation

The data for leaf length and SRL were square root transformed. Data for SLA, biomass (i.e. total biomass, leaf fresh weight, leaf dry weight, root fresh weight and root dry weight) and success ratio were log-transformed. After transformation the assumptions of ANOVA still were not met, which increases the risk of type 1 and 2 errors.

4.4.2 Nutrient solution and supply

The form in which nitrogen is provided (NH_4^+ , NO_3^-) for the plants could have affected the outcome of this research. In the nutrient solution we only applied NO_3^- , which is a simple inorganic form of N. Research by Hodge (2004) showed that when plants are in interspecific competition for organic patches containing a finite supply of mixed N sources root proliferation is important for N capture. When one of the factors is removed, in our case the mixed N sources, the importance of N capture might be obscured (Hodge, 2004). Thus the results, especially for the SRL, might be different when mixed N sources are used.

Temporal variation in nutrient supply and ratio could lead to a greater advantage for opportunistic species, because these species are more adaptable. In this experiment the various nutrient supply levels and ratios were constant. Species with a higher plastic response are expected to be a superior competitor in temporally variable environments.

4.4.3 Implications for ecosystem conservation

When assessing the effects of nutrients on ecological interactions and biodiversity it is important to look at the N:P supply ratio as well as the nutrient supply level, supporting the findings of Fujita (2010). Overall species perform best at intermediate to slightly N-limited conditions, indicating that N eutrophication could have a large impact on species performance and diversity.

The results show that on the short term, N specialists have a competitive advantage over the whole range of N:P supply ratios and nutrient supply levels. And that, on the short-term, phenotypic plasticity in this selection of traits (i.e. leaf length, SLA and SRL) is not important for explaining the success ratio of a species.

5.0 Conclusion

The study has shown that, under all circumstances, N-specialists perform better than P-specialists or opportunistic species. The competitive advantage of the N-specialists, in this experiment, could not be explained by phenotypic plasticity of the measured plant traits (i.e. leaf length, SLA and SRL). Furthermore species perform best at a intermediate (15) to low (5) N:P supply ratio. Under severe P-limitation the success ratio for all species is lowest. A positive relation between the N:P supply level and plant performance was found.

The interaction effects indicate the importance of looking at the effect of nutrient supply level as well as the N:P supply ratio on biomass and plant trait values. Interactions between N:P supply ratio and N:P supply level occurred for total biomass production, success ratio, leaf length and SLA. Indicating a different effect of N:P ratio per supply level. Species specific effects of N:P ratios on plant trait values were found.

Due to the short duration of the experiment only resource acquisition and use could affect the outcome. Future studies should also look at the long-term effects and thus include nutrient resorption and storage as possible explanations of plant species performance.

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Appendix 1.2: Recipe nutrient solution week 4-6

Week 4, 5 and 6 2,5 ml per pot, this for 2100 pots		Recepy Greenhouse experiment 2013		Nodig: 10500		10,5 liter	
Add in order as listed below							
Solution 1	NaNO3	N	AS LISTED BELOW	110	105		
Solution 2	NaH2PO4	P	AS LISTED BELOW	110	105		
Solution 3	KCl	K	AS LISTED BELOW	110	105		
Solution 4	CaCl	Ca	60 ml	110	105		
Solution 5	MgSO4	Mg - S	60 ml	110	105		
Solution 6	FeSO4	Fe - S	60 ml	110	105		
Solution 7	Ratjetoe	Cu, B, Mn, Zn, Mo	60 ml	110	105		
TOTAL FOR EVERY TREATMENT				420 ml	770	735	
				6,3	11,55	11,025	
N:P=1.67, low							
	MW	From concentrated solution (ml)	H2O to add (ml)	CHECK			
1	NaNO3	85	1,35802469135802	108,641975308642	110	Solution 4	CaCl Ca 110 ml
2	NaH2PO4	119,976	12,2222222222222	97,7777777777778	110	Solution 5	MgSO4 Mg - S 110 ml
3	KCl	74,55	55	55	110	Solution 6	FeSO4 Fe - S 110 ml
	inositol (CGH18O24P6)	660,024	Already in P solution...		0	Solution 7	Ratjetoe Cu, B, Mn, 110 ml
N:P=5, low							
	MW	From concentrated solution (ml)	H2O to add (ml)	CHECK			
1	NaNO3	85	2,35216776336514	107,647832236635	110	Solution 4	CaCl Ca 110 ml
2	NaH2PO4	119,976	7,05650329009543	102,943496709905	110	Solution 5	MgSO4 Mg - S 110 ml
3	KCl	74,55	55	55	110	Solution 6	FeSO4 Fe - S 110 ml
	inositol (CGH18O24P6)	660,024	Already in P solution...		0	Solution 7	Ratjetoe Cu, B, Mn, 110 ml
N:P=15, low							
	MW	From concentrated solution (ml)	H2O to add (ml)	CHECK			
1	NaNO3	85	4,07407407407407	105,925925925926	110	Solution 4	CaCl Ca 110 ml
2	NaH2PO4	119,976	4,07407407407407	105,925925925926	110	Solution 5	MgSO4 Mg - S 110 ml
3	KCl	74,55	55	55	110	Solution 6	FeSO4 Fe - S 110 ml
	inositol (CGH18O24P6)	660,024	Already in P solution...		0	Solution 7	Ratjetoe Cu, B, Mn, 110 ml
N:P=45, low							
	MW	From concentrated solution (ml)	H2O to add (ml)	CHECK			
1	NaNO3	85	7,05650329009543	102,943496709905	110	Solution 4	CaCl Ca 110 ml
2	NaH2PO4	119,976	2,35216776336514	107,647832236635	110	Solution 5	MgSO4 Mg - S 110 ml
3	KCl	74,55	55	55	110	Solution 6	FeSO4 Fe - S 110 ml
	inositol (CGH18O24P6)	660,024	Already in P solution...		0	Solution 7	Ratjetoe Cu, B, Mn, 110 ml
N:P=135, low							
	MW	From concentrated solution (ml)	H2O to add (ml)	CHECK			
1	NaNO3	85	12,2222222222222	97,7777777777778	110	Solution 4	CaCl Ca 110 ml
2	NaH2PO4	119,976	1,35802469135802	108,641975308642	110	Solution 5	MgSO4 Mg - S 110 ml
3	KCl	74,55	55	55	110	Solution 6	FeSO4 Fe - S 110 ml
	inositol (CGH18O24P6)	660,024	Already in P solution...		0	Solution 7	Ratjetoe Cu, B, Mn, 110 ml
N:P=1.67, medium							
	MW	From concentrated solution (ml)	H2O to add (ml)	CHECK			
1	NaNO3	85	4,07407407407408	105,925925925926	110	Solution 4	CaCl Ca 110 ml
2	NaH2PO4	119,976	36,6666666666667	73,3333333333333	110	Solution 5	MgSO4 Mg - S 110 ml
3	KCl	74,55	55	55	110	Solution 6	FeSO4 Fe - S 110 ml
	inositol (CGH18O24P6)	660,024	Already in P solution...		0	Solution 7	Ratjetoe Cu, B, Mn, 110 ml
N:P=5, medium							
	MW	From concentrated solution (ml)	H2O to add (ml)	CHECK			
1	NaNO3	85	7,05650329009543	102,943496709905	110	Solution 4	CaCl Ca 110 ml
2	NaH2PO4	119,976	21,1695098702863	88,8304901297137	110	Solution 5	MgSO4 Mg - S 110 ml
3	KCl	74,55	55	55	110	Solution 6	FeSO4 Fe - S 110 ml
	inositol (CGH18O24P6)	660,024	Already in P solution...		0	Solution 7	Ratjetoe Cu, B, Mn, 110 ml
N:P=15, medium							
	MW	From concentrated solution (ml)	H2O to add (ml)	CHECK			
1	NaNO3	85	12,2222222222222	97,7777777777778	110	Solution 4	CaCl Ca 110 ml
2	NaH2PO4	119,976	12,2222222222222	97,7777777777778	110	Solution 5	MgSO4 Mg - S 110 ml
3	KCl	74,55	55	55	110	Solution 6	FeSO4 Fe - S 110 ml
	inositol (CGH18O24P6)	660,024	Already in P solution...		0	Solution 7	Ratjetoe Cu, B, Mn, 110 ml
N:P=45, medium							
	MW	From concentrated solution (ml)	H2O to add (ml)	CHECK			
1	NaNO3	85	21,1695098702863	88,8304901297137	110	Solution 4	CaCl Ca 110 ml
2	NaH2PO4	119,976	7,05650329009543	102,943496709905	110	Solution 5	MgSO4 Mg - S 110 ml
3	KCl	74,55	55	55	110	Solution 6	FeSO4 Fe - S 110 ml
	inositol (CGH18O24P6)	660,024	Already in P solution...		0	Solution 7	Ratjetoe Cu, B, Mn, 110 ml
N:P=135, medium							
	MW	From concentrated solution (ml)	H2O to add (ml)	CHECK			
1	NaNO3	85	36,6666666666667	73,3333333333333	110	Solution 4	CaCl Ca 110 ml
2	NaH2PO4	119,976	4,07407407407407	105,925925925926	110	Solution 5	MgSO4 Mg - S 110 ml
3	KCl	74,55	55	55	110	Solution 6	FeSO4 Fe - S 110 ml
	inositol (CGH18O24P6)	660,024	Already in P solution...		0	Solution 7	Ratjetoe Cu, B, Mn, 110 ml
N:P=1.67, high							
	MW	From concentrated solution (ml)	H2O to add (ml)	CHECK			
1	NaNO3	85	12,2222222222222	97,7777777777778	110	Solution 4	CaCl Ca 110 ml
2	NaH2PO4	119,976	110	0	110	Solution 5	MgSO4 Mg - S 110 ml
3	KCl	74,55	55	55	110	Solution 6	FeSO4 Fe - S 110 ml
	inositol (CGH18O24P6)	660,024	Already in P solution...		0	Solution 7	Ratjetoe Cu, B, Mn, 110 ml
N:P=5, high							
	MW	From concentrated solution (ml)	H2O to add (ml)	CHECK			
1	NaNO3	85	21,1695098702863	88,8304901297137	110	Solution 4	CaCl Ca 110 ml
2	NaH2PO4	119,976	63,5085296108588	46,4914703891412	110	Solution 5	MgSO4 Mg - S 110 ml
3	KCl	74,55	55	55	110	Solution 6	FeSO4 Fe - S 110 ml
	inositol (CGH18O24P6)	660,024	Already in P solution...		0	Solution 7	Ratjetoe Cu, B, Mn, 110 ml
N:P=15, high							
	MW	From concentrated solution (ml)	H2O to add (ml)	CHECK			
1	NaNO3	85	36,6666666666667	73,3333333333333	110	Solution 4	CaCl Ca 110 ml
2	NaH2PO4	119,976	36,6666666666667	73,3333333333333	110	Solution 5	MgSO4 Mg - S 110 ml
3	KCl	74,55	55	55	110	Solution 6	FeSO4 Fe - S 110 ml
	inositol (CGH18O24P6)	660,024	Already in P solution...		0	Solution 7	Ratjetoe Cu, B, Mn, 110 ml
N:P=45, high							
	MW	From concentrated solution (ml)	H2O to add (ml)	CHECK			
1	NaNO3	85	63,5085296108588	46,4914703891412	110	Solution 4	CaCl Ca 110 ml
2	NaH2PO4	119,976	21,1695098702863	88,8304901297137	110	Solution 5	MgSO4 Mg - S 110 ml
3	KCl	74,55	55	55	110	Solution 6	FeSO4 Fe - S 110 ml
	inositol (CGH18O24P6)	660,024	Already in P solution...		0	Solution 7	Ratjetoe Cu, B, Mn, 110 ml
N:P=135, high							
	MW	From concentrated solution (ml)	H2O to add (ml)	CHECK			
1	NaNO3	85	110	0	110	Solution 4	CaCl Ca 110 ml
2	NaH2PO4	119,976	12,2222222222222	97,7777777777778	110	Solution 5	MgSO4 Mg - S 110 ml
3	KCl	74,55	55	55	110	Solution 6	FeSO4 Fe - S 110 ml
	inositol (CGH18O24P6)	660,024	Already in P solution...		0	Solution 7	Ratjetoe Cu, B, Mn, 110 ml

Appendix 1.3: Recipe nutrient solution week 7-9

Week 7,8,9 7,5 ml per pot, this for 2100 pots		Recepy Greenhouse experiment 2013		Nodig:		Nodig voor periode 3	
				10500	10,5 liter	15750	15,75 liter
Add in order as listed below						Extra	17,325
Solution 1	NaNO3	N	AS LISTED BELOW	110	105	165	170
Solution 2	NaH2PO4	P	AS LISTED BELOW	110	105	165	170
Solution 3	KCl	K	AS LISTED BELOW	110	105	165	170
Solution 4	CaCl	Ca	60 ml	110	105	165	170
Solution 5	MgSO4	Mg - S	60 ml	110	105	165	170
Solution 6	FeSO4	Fe - S	60 ml	110	105	165	170
Solution 7	Ratjetoe	Cu, B, Mn, Zn, Mo	60 ml	110	105	165	170
TOTAL FOR EVERY TREATMENT				770	735	1155	1190
				6,3	11,55	11,025	
						to use for recipe period 2 mliters	
N-P-1,67, low	M/W	From concentrated solution (ml)	H2O to add (ml)	CHECK			NaCl
1 NaNO3	85	2,037	162,963	165,000	Solution 4	CaCl	Ca 165 ml
2 NaH2PO4	119,976	18,333	146,667	165,000	Solution 5	MgSO4	Mg - S 165 ml
3 KCl	74,55	82,5	82,5	165	Solution 6	FeSO4	Fe - S 165 ml
inostol (GH18O24P6)		660,024	Already in P solution...		Solution 7	Ratjetoe	Cu, B, Mn, 165 ml
							pH meting
N-P-5, low		From concentrated solution (ml)	H2O to add (ml)				
1 NaNO3	85	3,528	161,472	165,000	Solution 4	CaCl	Ca 165 ml
2 NaH2PO4	119,976	10,585	154,415	165,000	Solution 5	MgSO4	Mg - S 165 ml
3 KCl	74,55	82,5	82,5	165	Solution 6	FeSO4	Fe - S 165 ml
inostol (GH18O24P6)		660,024	Already in P solution...		Solution 7	Ratjetoe	Cu, B, Mn, 165 ml
							pH meting
N-P-15, low		From concentrated solution (ml)	H2O to add (ml)				
1 NaNO3	85	6,111	158,889	165,000	Solution 4	CaCl	Ca 165 ml
2 NaH2PO4	119,976	6,111	158,889	165,000	Solution 5	MgSO4	Mg - S 165 ml
3 KCl	74,55	82,5	82,5	165	Solution 6	FeSO4	Fe - S 165 ml
inostol (GH18O24P6)		660,024	Already in P solution...		Solution 7	Ratjetoe	Cu, B, Mn, 165 ml
							pH meting
N-P-45, low		From concentrated solution (ml)	H2O to add (ml)				
1 NaNO3	85	10,585	154,415	165,000	Solution 4	CaCl	Ca 165 ml
2 NaH2PO4	119,976	3,528	161,472	165,000	Solution 5	MgSO4	Mg - S 165 ml
3 KCl	74,55	82,5	82,5	165	Solution 6	FeSO4	Fe - S 165 ml
inostol (GH18O24P6)		660,024	Already in P solution...		Solution 7	Ratjetoe	Cu, B, Mn, 165 ml
							pH meting
N-P-135, low		From concentrated solution (ml)	H2O to add (ml)				
1 NaNO3	85	18,333	146,667	165,000	Solution 4	CaCl	Ca 165 ml
2 NaH2PO4	119,976	2,037	162,963	165,000	Solution 5	MgSO4	Mg - S 165 ml
3 KCl	74,55	82,5	82,5	165	Solution 6	FeSO4	Fe - S 165 ml
inostol (GH18O24P6)		660,024	Already in P solution...		Solution 7	Ratjetoe	Cu, B, Mn, 165 ml
							pH meting
N-P-1,67, medium		From concentrated solution (ml)	H2O to add (ml)				
1 NaNO3	85	6,111	158,889	165,000	Solution 4	CaCl	Ca 165 ml
2 NaH2PO4	119,976	55,000	110,000	165,000	Solution 5	MgSO4	Mg - S 165 ml
3 KCl	74,55	82,5	82,5	165	Solution 6	FeSO4	Fe - S 165 ml
inostol (GH18O24P6)		660,024	Already in P solution...		Solution 7	Ratjetoe	Cu, B, Mn, 165 ml
							pH meting
N-P-5, medium		From concentrated solution (ml)	H2O to add (ml)				
1 NaNO3	85	10,585	154,415	165,000	Solution 4	CaCl	Ca 165 ml
2 NaH2PO4	119,976	31,754	133,246	165,000	Solution 5	MgSO4	Mg - S 165 ml
3 KCl	74,55	82,5	82,5	165	Solution 6	FeSO4	Fe - S 165 ml
inostol (GH18O24P6)		660,024	Already in P solution...		Solution 7	Ratjetoe	Cu, B, Mn, 165 ml
							pH meting
N-P-15, medium		From concentrated solution (ml)	H2O to add (ml)				
1 NaNO3	85	18,333	146,667	165,000	Solution 4	CaCl	Ca 165 ml
2 NaH2PO4	119,976	18,333	146,667	165,000	Solution 5	MgSO4	Mg - S 165 ml
3 KCl	74,55	82,5	82,5	165	Solution 6	FeSO4	Fe - S 165 ml
inostol (GH18O24P6)		660,024	Already in P solution...		Solution 7	Ratjetoe	Cu, B, Mn, 165 ml
							pH meting
N-P-45, medium		From concentrated solution (ml)	H2O to add (ml)				
1 NaNO3	85	31,754	133,246	165,000	Solution 4	CaCl	Ca 165 ml
2 NaH2PO4	119,976	10,585	154,415	165,000	Solution 5	MgSO4	Mg - S 165 ml
3 KCl	74,55	82,5	82,5	165	Solution 6	FeSO4	Fe - S 165 ml
inostol (GH18O24P6)		660,024	Already in P solution...		Solution 7	Ratjetoe	Cu, B, Mn, 165 ml
							pH meting
N-P-135, medium		From concentrated solution (ml)	H2O to add (ml)				
1 NaNO3	85	55,000	110,000	165,000	Solution 4	CaCl	Ca 165 ml
2 NaH2PO4	119,976	6,111	158,889	165,000	Solution 5	MgSO4	Mg - S 165 ml
3 KCl	74,55	82,5	82,5	165	Solution 6	FeSO4	Fe - S 165 ml
inostol (GH18O24P6)		660,024	Already in P solution...		Solution 7	Ratjetoe	Cu, B, Mn, 165 ml
							pH meting
N-P-1,67, high		From concentrated solution (ml)	H2O to add (ml)				
1 NaNO3	85	18,333	146,667	165,000	Solution 4	CaCl	Ca 165 ml
2 NaH2PO4	119,976	165,000	0,000	165,000	Solution 5	MgSO4	Mg - S 165 ml
3 KCl	74,55	82,5	82,5	165	Solution 6	FeSO4	Fe - S 165 ml
inostol (GH18O24P6)		660,024	Already in P solution...		Solution 7	Ratjetoe	Cu, B, Mn, 165 ml
							pH meting
N-P-5, high		From concentrated solution (ml)	H2O to add (ml)				
1 NaNO3	85	31,754	133,246	165,000	Solution 4	CaCl	Ca 165 ml
2 NaH2PO4	119,976	95,263	69,737	165,000	Solution 5	MgSO4	Mg - S 165 ml
3 KCl	74,55	82,5	82,5	165	Solution 6	FeSO4	Fe - S 165 ml
inostol (GH18O24P6)		660,024	Already in P solution...		Solution 7	Ratjetoe	Cu, B, Mn, 165 ml
							pH meting
N-P-15, high		From concentrated solution (ml)	H2O to add (ml)				
1 NaNO3	85	55,000	110,000	165,000	Solution 4	CaCl	Ca 165 ml
2 NaH2PO4	119,976	55,000	110,000	165,000	Solution 5	MgSO4	Mg - S 165 ml
3 KCl	74,55	82,5	82,5	165	Solution 6	FeSO4	Fe - S 165 ml
inostol (GH18O24P6)		660,024	Already in P solution...		Solution 7	Ratjetoe	Cu, B, Mn, 165 ml
							pH meting
N-P-45, high		From concentrated solution (ml)	H2O to add (ml)				
1 NaNO3	85	95,263	69,737	165,000	Solution 4	CaCl	Ca 165 ml
2 NaH2PO4	119,976	31,754	133,246	165,000	Solution 5	MgSO4	Mg - S 165 ml
3 KCl	74,55	82,5	82,5	165	Solution 6	FeSO4	Fe - S 165 ml
inostol (GH18O24P6)		660,024	Already in P solution...		Solution 7	Ratjetoe	Cu, B, Mn, 165 ml
							pH meting
N-P-135, high		From concentrated solution (ml)	H2O to add (ml)				
1 NaNO3	85	165,000	0,000	165,000	Solution 4	CaCl	Ca 165 ml
2 NaH2PO4	119,976	18,333	146,667	165,000	Solution 5	MgSO4	Mg - S 165 ml
3 KCl	74,55	82,5	82,5	165	Solution 6	FeSO4	Fe - S 165 ml
inostol (GH18O24P6)		660,024	Already in P solution...		Solution 7	Ratjetoe	Cu, B, Mn, 165 ml
							pH meting

Appendix 2: MATLAB code for analysing digital images

In order to determine the root length the digital images of the roots need to be analysed. These images were made with a scanner, first for one individual per scan. Later, to speed up the harvest, each scan contained two individuals. Therefore two slightly different codes in MATLAB were used for the analysis.

Rootscript enkel

%% Script to estimate Specific Root Length from line intercepts, using Tennant's method (J. Ecol 1975)

clear all

tic

% Load Image

X = imread('Pot0164PlantA','jpg'); % Loads the image (needs to be in same directory as this script)

X1=X(4000:9500,2700:6650,:);

%% Create a grid with specified resolution

[U V W] = size(X1); % Identify number of pixels in the image

l = 236; % Number of pixels for 0.5 cm width

w = 236; % Number of pixels for 0.5 cm length

GridX = zeros(U,V); % Initiate grid for Vertical lines

GridY = zeros(U,V); % Initiate grid for Horizontal lines

for i= 0:(U/l)

GridY(1+(i*l),:) = 1;
1+(i*l)

end

for j= 0:(V/w)

GridX(:,1+(j*w)) = 1;
1+(j*w)

end

Roots = min(1,(X1(:,:,1)<250)+(X1(:,:,2)<250)+(X1(:,:,3)<250)); %
Creates binary image of roots (=1) and background (=0)

RootImage = Roots; % Selects image area that contains the
roots (and nothing else)

RootMatrix1 = 2.*ones(U,V); % Create selected frame to show in
output

RootMatrix2 = RootImage; % Create selected frame to show in
output

GridXImage = GridX; % Adjust Grid for Vertical line mesh
accordingly

GridYImage = GridY; % Adjust Grid for Horizontal line mesh
accordingly

IntersectionX = (RootImage+GridXImage)==2; % Calculate where roots
intersect vertical grid

IntersectionY = (RootImage+GridYImage)==2; % Calculate where roots
intersect horizontal grid

```

%% Calculate Number of Intersections
[LY NUMY] = bxlabel(IntersectionY,4); % NUMY gives number of
intersections with vertical grid
[LX NUMX] = bxlabel(IntersectionX,4); % NUMX gives number of
intersections with horizontal grid
N = NUMY + NUMX; % Total Number of Intersections
R = (11/28).*N; % Gives the total Root Length

%% Show output
figure;subplot(221); imagesc(X);title(sprintf('The Specific Root Length is %g
cm', R),'FontSize',16)
subplot(223); imagesc(Roots+RootMatrix1);title('Frame Selected for Root
Length','FontSize',16)
subplot(224); imagesc(RootImage+IntersectionX+IntersectionY); title
('Intersections with Root highlighted','FontSize',16)
toc

```

RootScript

```

%% Script to estimate Specific Root Length from line intercepts, using Tennant's
method (J. Ecol 1975)

```

```
clear all
```

```
tic
```

```
% Load Image
```

```
X = imread('Pot0002PlantsAandC','jpg'); % Loads the image (needs to be
in same directory as this script)
```

```
X1=X(1990:5150,2000:8300,:);
```

```
%% %% Create a grid with specified resolution
```

```
[U V W] = size(X1); % Identify number of pixels in the image
```

```
l = 236; % Number of pixels for 0.5 cm width
```

```
w = 236; % Number of pixels for 0.5 cm length
```

```
GridX = zeros(U,V); % Initiate grid for Vertical lines
```

```
GridY = zeros(U,V); % Initiate grid for Horizontal lines
```

```
for i= 0:(U/l)
```

```
GridY(1+(i*l),:) = 1;
1+(i*l)
```

```
end
```

```
for j= 0:(V/w)
```

```
GridX(:,1+(j*w)) = 1;
1+(j*w)
```

```
end
```

```
Roots = min(1,(X1(:,,1)<250)+(X1(:,,2)<250)+(X1(:,,3)<250)); %
Creates binary image of roots (=1) and background (=0)
```

```
RootImage = Roots; % Selects image area that contains the
roots (and nothing else)
```

```
RootMatrix1 = 2.*ones(U,V); % Create selected frame to show in
```

```

output
RootMatrix2      = RootImage;           % Create selected frame to show in
output
GridXImage       = GridX;               % Adjust Grid for Vertical line mesh
accordingly
GridYImage       = GridY;               % Adjust Grid for Horizontal line mesh
accordingly
IntersectionX     = (RootImage+GridXImage)==2; % Calculate where roots
intersect vertical grid
IntersectionY     = (RootImage+GridYImage)==2; % Calculate where roots
intersect horizontal grid

%% Calculate Number of Intersections
[LY NUMY]        = bwlabel(IntersectionY,4); % NUMY gives number of
intersections with vertical grid
[LX NUMX]        = bwlabel(IntersectionX,4); % NUMX gives number of
intersections with horizontal grid
N                = NUMY + NUMX;         % Total Number of Intersections
R                = (11/28).*N;         % Gives the total Root Length

%% Show output
figure;subplot(221); imagesc(X);title(sprintf('The Root Length is %g cm',
R),'FontSize',16)
subplot(223); imagesc(Roots+RootMatrix1);title('Frame Selected for Root
Length','FontSize',16)
subplot(224); imagesc(RootImage+IntersectionX+IntersectionY); title
('Intersections with Root highlighted','FontSize',16)
toc

X2=X(7150:10940,2000:8300,:);
% %% Create a grid with specified resolution
[U V W]          = size(X2);           % Identify number of pixels in the image
l                = 236;                 % Number of pixels for 0.5 cm width
w                = 236;                 % Number of pixels for 0.5 cm length
GridX            = zeros(U,V);         % Initiate grid for Vertical lines
GridY            = zeros(U,V);         % Initiate grid for Horizontal lines
for i= 0:(U/l)
    GridY(1+(i*l),:) = 1;
    1+(i*l)
end
for j= 0:(V/w)
    GridX(:,1+(j*w)) = 1;
    1+(j*w)
end

Roots            = min(1,(X2(:,:,1)<250)+(X2(:,:,2)<250)+(X2(:,:,3)<250)); %
Creates binary image of roots (=1) and background (=0)
RootImage        = Roots;              % Selects image area that contains the
roots (and nothing else)

```

```

RootMatrix1    = 2.*ones(U,V);      % Create selected frame to show in
output
RootMatrix2    = RootImage;        % Create selected frame to show in
output
GridXImage     = GridX;            % Adjust Grid for Vertical line mesh
accordingly
GridYImage     = GridY;            % Adjust Grid for Horizontal line mesh
accordingly
IntersectionX   = (RootImage+GridXImage)==2; % Calculate where roots
intersect vertical grid
IntersectionY   = (RootImage+GridYImage)==2; % Calculate where roots
intersect horizontal grid

%% Calculate Number of Intersections
[LY NUMY]      = bwlabel(IntersectionY,4); % NUMY gives number of
intersections with vertical grid
[LX NUMX]      = bwlabel(IntersectionX,4); % NUMX gives number of
intersections with horizontal grid
N              = NUMY + NUMX;         % Total Number of Intersections
R              = (11/28).*N;         % Gives the total Root Length

%% Show output
figure;subplot(221); imagesc(X);title(sprintf('The Root Length is %g cm',
R),'FontSize',16)
subplot(223); imagesc(Roots+RootMatrix1);title('Frame Selected for Root
Length','FontSize',16)
subplot(224); imagesc(RootImage+IntersectionX+IntersectionY); title
('Intersections with Root highlighted','FontSize',16)
toc

```

Appendix 3: post hoc test

The post hoc test for the success ratio, ANOVA split by N:P ratio. It shows that N-specialists have the significantly highest success ratio for all N:P supply ratios while for opportunists the success ratio is lowest.

NP_ratio=1,7

Tukey HSD^{a,b,c}

species	N	Subset			
		1	2	3	4
O1	47	-2,4171			
O2	46		-1,7133		
P1	24		-1,2210	-1,2210	
P2	24			-,6470	-,6470
N2	24			-,6467	-,6467
N1	24				-,5458
Sig.		1,000	,193	,080	,997

Means for groups in homogeneous subsets are displayed.
Based on observed means.

The error term is Mean Square(Error) = ,646.

- Uses Harmonic Mean Sample Size = 28,615.
- The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
- Alpha =

NP_ratio= 5

Tukey HSD^{a,b,c}

species	N	Subset		
		1	2	3
O1	45	-2,0646		
O2	48	-1,8618		
P1	24		-,6626	
P2	24		-,4108	
N2	22		-,0377	-,0377
N1	26			,4045
Sig.		,954	,086	,411

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,780.

- Uses Harmonic Mean Sample Size = 28,530.
- The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
- Alpha =

NP_ratio=15

Tukey HSD^{a,b,c}

species	N	Subset		
		1	2	3
O1	49	-2,5993		
O2	42	-2,4080		
P1	23		-1,4714	
P2	23		-,9821	
N2	22			-,0831
N1	26			,2450
Sig.		,985	,530	,862

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1,148.

- a. Uses Harmonic Mean Sample Size = 27,895.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
- c. Alpha =

NP_ratio=45

Tukey HSD^{a,b,c}

species	N	Subset		
		1	2	3
O1	44	-4,2494		
O2	40	-3,7596		
P1	22		-2,5332	
P2	26		-2,4185	
N1	24			-1,1010
N2	24			-,6259
Sig.		,294	,996	,328

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,761.

- a. Uses Harmonic Mean Sample Size = 27,910.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
- c. Alpha =

NP_ratio=135

Tukey HSD^{a,b,c}

species	N	Subset				
		1	2	3	4	5
O2	35	-5,5393				
O1	44		-4,7390			
P2	24			-3,7654		
P1	24				-2,9064	
N1	24					-1,9129
N2	24					-1,6439
Sig.		1,000	1,000	1,000	1,000	,788

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,596.

- a. Uses Harmonic Mean Sample Size = 27,527.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
- c. Alpha =

The post hoc test of the SLA per species, one way ANOVA split by N:P ratio.

NP_ratio=1,7

Tukey HSD^{a,b}

species	N	Subset for alpha = 0.05
		1
P1	46	5,0472
N1	47	5,0558
P2	47	5,1401
O1	63	5,1760
N2	45	5,2447
O2	63	5,2886
Sig.		,173

Means for groups in homogeneous subsets are displayed.

- a. Uses Harmonic Mean Sample Size = 50,735.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

NP_ratio=5

Tukey HSD^{a,b}

species	N	Subset for alpha = 0.05		
		1	2	3
O1	57	4,8559		
N1	45	4,8872	4,8872	
P1	46	5,0751	5,0751	5,0751
N2	45		5,2566	5,2566
O2	57			5,2713
P2	48			5,3020
Sig.		,558	,060	,520

Means for groups in homogeneous subsets are displayed.

- a. Uses Harmonic Mean Sample Size = 49,138.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

NP_ratio=15

Tukey HSD^{a,b}

species	N	Subset for alpha = 0.05	
		1	2
N1	48	4,9949	
P1	44	5,2070	5,2070
O2	55	5,2623	5,2623
O1	58	5,2625	5,2625
P2	47	5,2688	5,2688
N2	45		5,3630
Sig.		,162	,749

Means for groups in homogeneous subsets are displayed.

- a. Uses Harmonic Mean Sample Size = 48,987.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

NP_ratio=45

Tukey HSD^{a,b}

species	N	Subset for alpha = 0.05		
		1	2	3
P1	43	4,9551		
N1	46	4,9901	4,9901	
O2	50	5,0871	5,0871	5,0871
O1	43	5,1856	5,1856	5,1856
N2	44		5,2413	5,2413
P2	48			5,3305
Sig.		,146	,086	,106

Means for groups in homogeneous subsets are displayed.

- a. Uses Harmonic Mean Sample Size = 45,520.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

NP_ratio=135

Tukey HSD^{a,b}

species	N	Subset for alpha = 0.05		
		1	2	3
N1	41	4,9097		
P1	46	5,0455	5,0455	
O2	34	5,1567	5,1567	5,1567
O1	40	5,1906	5,1906	5,1906
N2	47		5,3161	5,3161
P2	45			5,3667
Sig.		,109	,135	,391

Means for groups in homogeneous subsets are displayed.

- a. Uses Harmonic Mean Sample Size = 41,655.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.