University of Tartu

Faculty of Science and Technology

Institute of Ecology and Earth Sciences

Department of Botany

Laura Puura

# Plants' morphological plasticity and how it affects species diversity in a plant community. A four-year plucking experiment in Laelatu wooded meadow

Biology and Eco-innovation

Master's thesis (30 EAP)

Supervisor: Hanna Kaarin Hermlin

Tartu 2023

## Taimede morfoloogilise plastilisuse mõju taimekoosluse liigirikkusele. Nelja-aastane kitkumiskatse Laelatu puisniidul

Taimed ei saa keskkonnamuutuste eest põgeneda, vaid peavad leidma viise, kuidas võimalikult hästi oma ümbrusega toime tulla. Üheks selliseks viisiks on plastilisus – organismi võime luua erinevates keskkondades just sinna sobiv fenotüüp. Lisaks indiviidi tunnuste muutmisele avaldab plastilisus mõju ka taimede kooseksisteerimisele, kuid mõju suunas jäävad senised uurimistulemused eriarvamustele: võib toimuda nii koosluse liigirikkuse kasv kui ka ühe liigi domineerimine teiste üle. Käesoleva töö eesmärk oli uurida taimede lehtede arvu ja lehe eripinna (SLA) plastilisuse mõju liigirikkusele. Töö kaks hüpoteesi – 1) suurem SLA plastilisus vähendab koosluse liigirikkust; 2) suurem lehtede arvu plastilisus suurendab koosluse liigirikkust – ei leidnud tõestust. Oluline koosmõju ilmnes ruudu biomassi ja liigirikkuse vahel, aga plastilisuse mõju liigirikkusele veel avalduda ei ole jõudnud.

Märksõnad: taimede morfoloogiline plastilisus, liikide kooseksisteerimine, konkurents, kooseksisteerimise mehhanismid. CERCS kood: B270- Taimeökoloogia

## Plants' morphological plasticity and how it affects species diversity in a plant community. A four-year plucking experiment in Laelatu wooded meadow

Plants cannot escape changing environmental conditions but must find ways to cope with them. One can be plasticity – the ability to produce the best-fitting phenotype for all environments. In addition to benefiting individuals, plasticity can affect plant coexistence, however, the scientific findings disagree about the direction of the impact: whether plasticity promotes species richness or impedes it with the appearance of dominants. The purpose of this thesis was to study the influence of leaf number and specific leaf area plasticity (SLA) on species richness. Two hypotheses – 1) greater plasticity in SLA decreases species richness; 2) greater plasticity in leaf number increases species richness – did not find proof. Important interaction appeared between the quadrat's biomass and species richness, but plasticity's influence on species richness has not yet become evident.

Keywords: plant morphological plasticity, species diversity, species coexistence, competition, coexistence mechanisms. CERCS Code: B270- Plant ecology

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# 1. Introduction

It is evident that still not enough is known about plants and the way they interact and affect each other. Some scientists believe that plant communities are individualistic – composed of species that share the same resource acquirements and happen to be dispersed together into the same place forming a community (Whitfield, 2002). On the other hand, some are certain that plants are interdependent, depending on other plants through positive and indirect interactions, that the formation and persistence of a community relies on species alleviating the environment for others (Callaway, 2007). The truth might be even more perplexing as more information is emerging about complex interactions, with plants being able to recognize their neighbors and tell apart relatives from strangers.

Another interesting phenomenon about plants is their ability to be plastic. Being able to change in response to variability in the environment should be beneficial to all organisms, especially to plants as they are not able to relocate into a better-suiting environment (Sultan, 2000). But studying the effect of plasticity on the plant community has shown contrary results: sometimes plant stands with higher plasticity promote species richness (Lepik & Zobel, 2015; Turcotte & Levine, 2016), but other times highly plastic plants become superior competitors, and dominate over others (Ashton, Miller, Bowman & Suding, 2010; Xu et al., 2022).

The aim of this thesis is to investigate small-scale plant-on-plant interactions, mainly competition and facilitation, and the effect of plasticity on the plant community. The first chapter gives an overview of how different plant species can coexist and what is the role of intra-, and interspecific competition, facilitation, and kin recognition. In the second chapter, plant plasticity is added to the community structuring mechanisms, and its role in alleviating or hindering diversity is discussed. The most important part, however, is a four-year permanent plot experiment on Laelatu wooded meadow. This plasticity-manipulating experiment explores plant leaf number and specific leaf area (SLA) plasticity and their function in creating species richness.

# 2. Interactions between plants

The environment – both abiotic and biotic – affects plants. With more focus on the climate change, growing body of evidence accumulates on the effects of increased air temperature, frequent droughts and floods, and the possible coping mechanisms for plants. However, plants also interact with each other – this phenomenon is quite clear to us from extensive research done during the last century (Mack & Harper, 1977). Although the mechanisms have not been easy to understand since a plant has no nervous system or visible sensory organs to "see" what is happening around it. Moreover, it is hard to predict the outcome of the interaction between two or more plant individuals (or species), who react to the availability of light and other resources while, at the same time, competing for those limited resources. This is why plant-on-plant interactions still mesmerize scientists and inspire them to contribute to this field.

An important concept shaping the outcome of interactions between plants is the niche. A species' niche represents the ecological needs of given species. It describes the species' requirements for different resources (light, water, minerals, space, etc.) for it to be able to persist and expresses the species' ecological role in that environment. Including the role it has for other species, as a niche contains all interactions one species has with abiotic and biotic environments (Polechová & Storch, 2008). Through negative and positive interactions (competition and facilitation), a species impacts its neighbors' abundance and viability – whether by depleting resources from the soil and changing the plant-soil feedback (Bennett & Klironomos, 2019) or one species making an otherwise inhabitable environment suitable for others (Bertness & Callaway, 1994). But when species' demands from the environment are too similar in a way, that a substantial part of their niches overlaps, competition for shared resources peaks with the competitive exclusion of a weaker competitor (Hutchinson, 1959). The weaker competitor typically exhibits a slower growth rate or is incapable to persist under low resource availability (Tilman, 1982). Yet focusing on processes functioning on a small scale, there are multiple mechanisms that allow even similar species to coexist.

## 2.1. Competition and coexistence mechanisms

Plant-plant interactions can lead to coexistence or competitive exclusion. Modern coexistence theory proposes two types of mechanisms – equalizing and stabilizing – that could lead to a short-term or stable plant coexistence. When one plant is better at using shared resources than its neighbor, chances are it will outcompete the latter (Turcotte & Levine, 2016). Equalizing mechanisms help to minimize differences in plants' competitive abilities, that is fitness differences, that drive competitive exclusion in the first place (Chesson, 2000). Still, equalizing processes alone are not able to ensure stability for a community because, in the case of two species, one of them can still be better at enduring minimal level of resources. Therefore, the purpose of equalizing mechanisms is to postpone competitive exclusion and together with stabilizing processes bring about stable coexistence (Chesson, 2000). Intraspecific aggregation could be seen as an equalizing mechanism where competition between a batch of conspecifics with low competitive ability and heterospecifics with high competitive ability will take place only along the borders of the batch and therefore it will take longer to eliminate the weaker competitor (Barot & Gignoux, 2004).

For stable coexistence, a species needs an opportunity to recover its density after a perturbation. Stabilizing mechanisms create room for different species to invade a community by increasing intraspecific competition in relation to interspecific competition and are benefitted by heterogeneity and variability in space and time (Chesson, 2000; Barot & Gignoux, 2004). A heterogeneous environment makes it possible to fit multiple species into a small area as a species is the best competitor only in a fraction of space with the most suited environmental conditions. While conspecifics share a niche, competition among them will be higher than between different species, allowing a newcomer to experience lower competition until its density rises (Barot & Gignoux, 2004). Therefore, intraspecific aggregation can also be seen as a stabilizing force, where the aggregated species suppress its own growth through a similar need for resources to a higher extent than its heterospecific neighbors'. This effect is known as negative conspecific density dependence, where a species' population growth rates decrease as this species becomes more common in a community. This is thought to be one mechanism that enables tropical rainforests to have such high species richness with exceptionally high numbers of rare species (LaManna et al., 2017).

But research shows contrary results: no significant difference between intra- and interspecific competition was found for two co-occurring perennial grass species (Aguiar, Lauenroth & Peters, 2001) nor was it found for coexisting grassland annuals from a six-species experiment (Leger & Espeland, 2010). On the other hand, an extensive meta-analysis showed that in various ecosystems, intraspecific competition is four to five times greater than interspecific competition. The difference was especially pronounced in grasslands

(Adler *et al*., 2018). The same study also found that manipulative greenhouse experiments tend to show more similar values for intra- and interspecific competition than field observational studies. To further elucidate this finding, fixed settings in a greenhouse and experiments with manipulated densities reject the effects of interaction chains that could mediate interspecific competition and allow coexistence (plant-soil interactions, higher-order interactions). It seems that the theory of high intraspecific competition being the force allowing multiple species to coexist is accurate and results showing otherwise do not account for actual processes happening in nature.

Adler, Ellner and Levine (2010) found that stabilizing effects overperformed fitness differences more than was needed for coexistence. The study used data from a long-term quadrat experiment for modeling species interactions. When they removed niche differences, that accounted for the stabilizing force, from a model with four species, one of the species with the highest fitness slowly over time outcompeted the others. On the other hand, the same niche differences that differentiate species and work as a stabilizing force can contribute to greater fitness differences between species and lead to competitive exclusion. For example, later phenology is one of the traits that can make one species a superior competitor, but later phenology can also promote the emergence of stabilizing niche differences between species (minimized competition for pollinators). While later phenology alone can give competitive dominance to one species over another, Kraft, Godoy & Levine (2015) found no single trait, that could account for stabilizing niche differences. Different traits across ecological niche space, like phenology, seed size, and specific root length, had to operate together for a stabilizing effect. Consequently, not all trait differences between species are stabilizing in essence, but multiple trait differences together in addition to heterogeneity in space and time help species to coexist.

## 2.2. The importance of facilitation

During the last century, competitive interactions have been the focus of plant-plant interactions. Even nowadays competition, especially interspecific competition, is thought to be the main force driving community assembly (Götzenberger *et al.*, 2012). However, more and more information is accumulating about positive interactions being no less of great importance (Callaway, 2007). Through resource acquisition, which varies among species, plants change the environment locally, affecting the survival of other species and the whole composition of the plant community (Barot & Gignoux, 2004). From there, a shortage of common recourses can aggravate competition, or the creation of shade through canopy cover can make it possible for a species to occupy a space that had excessive solar radiation before.

Facilitation is a positive interaction between closely growing plants, where just the presence of one neighbor can enhance the growth and survival of another. Contrary to competition, it alleviates the growth environment for plants. Usually, facilitation does not happen on purpose, it is not the species' intention to altruistically help another species. Facilitation happens when one species modifies the environment whether through resource acquisition, shading, or creation of suitable substrates, and makes it more fitting to others (Callaway, 2007). Facilitation is found in intra- and interspecific interactions but more profoundly between interspecifics. This is supporting species diversity by alleviating interspecific interactions more in relation to intraspecific interactions and therefore creating a stabilizing effect (Adler et al., 2018). Most examples of facilitation come from stressful environments, where the increase in stressful conditions creates more opportunities for facilitation within (Fajardo & McIntire, 2011) and between species (Bertness & Callaway, 1994).

Called the "stress gradient hypothesis," research shows that interactions can shift from competitive to facilitative with the increasing harshness of environmental conditions (Bertness & Callaway, 1994; Chu, Maestre, Xiao, Weiner, Wang, Duan & Wang, 2008; García-Cervigón, Gazol, Sanz, Camarero & Olano, 2013). Both intra- and interspecific competition can decrease with heightened stress – conspecifics will benefit from growing in clusters (positive density-dependence) (Goldenheim, Irving & Bertness, 2008; Fajardo & McIntire, 2011), and heterospecifics will take advantage of the positive interaction caused by their neighbor (García-Cervigón, Gazol, Sanz, Camarero & Olano, 2013). This applies from intermediate to high-stress levels, where stress-intolerant species benefit from positive interactions, whereas in severely stressful conditions solely facilitative effects might not be enough for survival (Michalet *et al*., 2006). However, on the other side of the gradient with benign conditions, conspecific aggregation will be an unfavorable choice when the intraspecific competition becomes too intense (negative density-dependence) (Goldenheim, Irving & Bertness, 2008), and fast-growing competitive species will dominate over slow-growing stress-tolerant species, reducing local species richness (Michalet *et al*., 2006). There will always be competition within species and between co-occurring species, but whether the biotic alleviating effects can compensate for stressful conditions will determine the net outcome and balance between facilitation and competition.

Kin selection – a situation where relatives seem to be altruistic, sharing space and resources among themselves to increase their inclusive fitness (Hamilton, 1964) – can make plant–plant interactions more complex and entangled. Kin recognition with cooperation is one way to alleviate detrimental intraspecific competition between kins (West, Pen & Griffin, 2002). As with facilitation, it is hard to determine the presence of kin recognition in nature – measuring an individual's inclusive fitness is necessary, not just the mean fitness of the interacting species (Ehlers & Bilde, 2019). Closely growing individuals of the same species might have evolved with the ability to recognize their relatives and minimize the competition among themselves, but whether it will be enough to overcome the competitive effect of aggregation depends on specific conditions (West, Pen & Griffin, 2002; Ehlers & Bilde, 2019). Clonal and self-pollinating plants are more prone to kin cooperation than outbreeders, supporting the theory that closely growing conspecifics have mechanisms to alleviate competition (Subrahmaniam, Roby & Roby Roux, 2021). This behavior also challenges coexistence between species as intraspecific competition diminishes.

Although most of the research on facilitative effects has been done in stressful environments, such positive interactions are ubiquitous and relevant. For example, pioneer species modify novel environments to be suitable for others, they protect frail species from wind and excess radiation, and neighbors can increase accessibility to water through the hydraulic lift and help to keep the moisture in the soil (Callaway, 2007). Moderate shade can strongly facilitate plant growth (greater mass) and lower seedling mortality in grassland communities (Semchenko, Lepik, Götzenberger & Zobel, 2012). Also in grasslands, deep-rooted *Quercus douglasii* individuals have a positive effect on understory biomass – trees fertilize the soil with litter input and as they access the deeper water table, they do not compete for water with species growing beneath them (Callaway, Nadkarni & Bruce, 1991). Positive interactions are significant because they promote multispecies coexistence, especially when superior competitors foster the growth of other species (Gross, 2008). The highest numbers of species richness appear in the intermediate stress levels, with facilitation being the net outcome of species interactions (Michalet et al., 2006). That is why facilitation, besides competition, should be considered when analyzing processes that create a particular community.

# 3. Plasticity

Phenotypic plasticity, the ability of a genotype to produce different phenotypes in response to the environment (Bradshaw, 1965), has an important role in an individual's survival (Schneider, 2022) and plant community assembly (Callaway, Pennings & Richards, 2003). As a reaction to the environment, plasticity can be triggered by changes in abiotic components like the availability of light (Lepik & Zobel, 2015), the amount of water (Wang & Callaway, 2021), and nutrients in the soil (Schiffers, Tielbörger, Tietjen & Jeltsch, 2011) the mean annual temperature (Stotz, Salgado-Luarte, Escobedo, Valladares & Gianoli, 2021), and so on. However, more interestingly, plastic responses appear against biotic interactions too – in response to plant-on-plant interactions like competition and facilitation, as discussed above.

All organisms are somewhat plastic and show variation in their traits (Sultan, 2000). For plants being able to change is most utterly important as they cannot "walk" away when environmental conditions should change. Variations in the abiotic environment – fluctuations in light and water availability as well as nutrient composition – or in the biotic environment – the presence of herbivory, parasitism, or mutualistic relations with neighbors – bring about plastic changes in plants (Callaway, Pennings & Richards, 2003). To prevail this variability in the surroundings, plants are composed of semi-individualistic modules, that all can change during a plant's lifespan and reiterate the individual if needed (Gilroy & Trewavas, 2001). Different plant modules above- and belowground are discrepant in their plasticity in response to an environmental factor. Moreover, the modules are simultaneously affected by multiple environmental factors (De Kroon, Huber, Stuefer, Van Groenendael, 2005). Hence, the plasticity of the whole plant is composed of its separate models' responses to the environment and the effect caused by interactions between the modules themselves (Schneider, 2022).

Plasticity occurs mostly in low to intermediate stress levels, where environmental conditions are near optimal. Plants can have variability in their traits to try to fit in the best way possible with the environment. But in highly stressful environments, specialists, who have adapted to this limited environment, prevail over generalists, as these harsh conditions do not allow for trait variability (Brooker *et al.*, 2022). Another way, how the environment constrains plastic responses, is through its predictability. Plastic responses are adaptive when a plant can detect informative signals from the environmental change and then create the most fitting phenotype possible. However, when the environment should change too unpredictably, cues can be unreliable and therefore create a wrong phenotype (Schneider, 2022).

In theory, talking about plastic plants while only considering the plasticity of its few modules (root plasticity for example) is justifiable, but empirically can be rather useless. If plasticity in response to light availability is the aspect under observation, high plasticity of roots will not guarantee that the aboveground parts are as plastic (Hermlin & Zobel, unpublished). And more, the environmental stimulus that triggers plasticity is the key component for making sense of plastic responses. Roots might be more plastic than on average in response to the concentration of nitrogen, but their reaction to light availability is usually overlooked, since shoots are more affected by light than roots. Still, sometimes great changes in the belowground environment can influence leaf traits in addition to roots and vice versa as the plant as a whole is adapted to living in a certain environment. Waterlogging made plant leaves express plastic changes even more than shading (Dostál, Fischer, Chytrý & Prati, 2017), but because no belowground traits were measured, the impact size to root traits in comparison with leaf traits remains unknown. On some level, it is known that cells in one plant module have information on how the environment is affecting the cells in other modules (Gilroy & Trewavas, 2001). When roots sense water deficiency in the soil, the signal travels through leaves where an important component of the plant's drought stress response – abscisic acid – is synthesized (Takahashi, Kuromori, Urano, Yamaguchi-Shinozaki & Shinozaki, 2020). The roots of seedlings, that are grown in total darkness, stay much shorter compared to seedlings that are grown in light (van Gelderen, Kang & Pierik, 2018). Therefore, the plasticity value of the whole plant could have a meaning, when compared between species, which is important considering plant coexistence.

There are plenty of examples of plant phenotypic plasticity. A well-known case of a plant's reaction against light availability is the shade avoidance response. In the case of shading, plants lengthen their petioles and stem to rise again to sunlight, orientate shoots towards canopy gaps, reduce branching or tillering (by mediating bud dormancy), reduce biomass allocation to roots and leaf mass per area, and downregulate defense pathways (Ballaré, Scopel, Jordan & Vierstra, 1994; Fernández-Milmanda & Ballaré, 2021). When water stress should occur in the soil, plastic changes include shortening of the roots and increasing their diameter in addition to the reduction in both shoot and root mass (Cai et al., 2017). With competition in resource acquisition, root plasticity can minimize belowground competition by avoiding the overlap of plants' roots (Schiffers *et al.,* 2011). Some morphological changes are relatively easy to measure, but variability in organisms can be found on physiological, phenological, behavioral, and anatomical scales, and are much harder to grasp and consider.

As ubiquitous as plasticity may seem, not every plastic response to the environment is adaptive. Plasticity may be adaptive if through plastic changes a plant increases its fitness across a range of environments, where selective pressures differ – the plant is favored and has higher fitness than it would have without plastic responses (Dorn, Pyle & Schmitt, 2000). In the case of two environments – high and low density stands – *Impatiens capensis* Meerb. (*Balsaminacea*) had an adaptive plastic response to crowding: taller plants had higher fitness in dense stands, while shorter plants were fitter in less crowded stands (Dudley & Schmitt, 1996). Competitive superiority can also shift from having fast growth and high resource usage in rainfall conditions to high water use efficiency in drought conditions (Pérez-Ramos, Matías, Gómez-Aparicio & Godoy, 2019). But a lot of the time plastic modifications get mistaken as adaptive even when they are not under selection (Bonser, 2021). All observed plasticity is not adaptive (not related to fitness) as plasticity that enhances survival is not always related to having higher fecundity when compared to a nonplastic phenotype (Arnold, Nicotra & Kruuk, 2019; Brooker *et al*., 2022).

Plasticity can be even maladaptive (lowering fitness) if the plant misunderstands the environment and creates a wrong phenotype, or the right one but with too big of a lag in time. As plastic changes are costly and require a precise prediction of environmental fluctuations, they are difficult and risky to succeed in. The cost represents the energy a plant must put into creating sensory organs (phytochromes for example) and using them (DeWitt, Sih & Wilson, 1998). When reacting to being shaded, plants must cope with the risks of heightened solar radiance, evaporation, and exposure to herbivory (Fernández-Milmanda & Ballaré, 2021). In the case of a mismatch between the plant and the environment, the cost of being plastic prevails over the benefit of what the plastic change brought with it, lowering the plant’s fitness in comparison to the state before the plastic response (Valladares, Gianoli & Gómez, 2007). This is why all organisms are not perfectly plastic and can’t succeed in all environments.

## 3.1. The conflicting nature of plasticity for species coexistence

Plasticity can influence both intraspecific and interspecific competition (Callaway, Pennings & Richards, 2003). The outcome – coexistence or competitive exclusion – depends on whether the result of plastic responses keeps intraspecific competition greater than interspecific, allowing coexistence, or increases interspecific competition above the intraspecific competition, excluding the weaker competitor (Chesson, 2000). Turcotte & Levine (2016) show that if plasticity allows niche differentiation that acts as a stabilizing force by allowing a species to grow its abundance from low to high, it can promote stable coexistence when combined with similar (or minimized) competitive abilities between species. However, competitive exclusion should occur when one species becomes relatively less or more fit because of plastic changes (Turcotte & Levine, 2016).

A lot of the time niche differentiation and trait divergence that happen through plastic responses exhibit improved coexistence between species (Chesson, 2000) or greater productivity (Burns & Strauss, 2012). These results arise from competition experiments between species pairs, where differences in species' traits are revealed when species are grown alone or with a heterospecific, and from wider community experiments, where higher-order interactions and environmental variability add a layer of complexity. Competition experiments with two plant species, *Lemna minor* and *Spirodela polyrhiza*, indicate that plasticity can change species' specific leaf area, making it possible for *L. minor* to invade *S. polyrhiza*'s colony and the other way around. This experiment shows how plasticity makes it possible for two similar species to coexist and that a new species could invade a community by altering its trait values and lowering interspecific competition in relation to intraspecific (Hess, Levine, Turcotte & Hart, 2022). With intraspecific variability in SLA and plant height, one species can inhabit different environmental conditions – highly flooded areas select for higher SLA and height than less flooded areas. Therefore, one species can contribute to species richness in multiple communities (Jung, Violle, Mondy, Hoffmann & Muller, 2010).

Experiments have revealed greater interspecific differences in plant height and SLA in species mixtures than in monocultures (Zuppinger-Dingley, Schmid, Petermann, Yadav, De Deyn & Flynn, 2014). In a multispecies community containing various growth forms, creeping reptant species shortened while rosulate species lengthened their leaves, creating niche differences for light-acquisition traits (Lipowsky *et al.*, 2015). In a community with different space-colonizing strategies, horizontally spreading species minimized the variation in their lengths as there was no point in competing with taller plants, while some vertically spreading plants grew even higher and lost their shorter individuals. In the end, plants' heights were distributed more evenly on the vertical scale, and their niches became more different (Meilhac, Deschamps, Maire, Flajoulot & Litrico, 2020). For two freshwater ciliate species, more significant plastic responses on mean trait values (biomass, cell shape, dispersal ability) occurred in a more stressful environment (higher salinity), but the responses were altered by competition: one species significantly increased the dispersal abilities, decreased its biomass, and became longer as the other became significantly more round (Govaert, Gilarranz & Altermatt, 2021).

In addition to greater niche differences, grassland community studies detect that higher shoot plasticity minimizes fitness differences between species. Community composed of plants having a more remarkable shoot plasticity to light availability revealed denser stands with higher species richness and more even canopy height (Lepik, Liira & Zobel, 2005). Without great differences in plant height, competition for light will get more symmetric, meaning, one plant will not be able to gain bigger success through competing for light than the others. In addition to ramet density, plasticity can even have a direct positive effect on species richness (Lepik & Zobel, 2015). The link between shoot plasticity and species richness was novel, but it has been shown before that morphological plasticity can lower the variance in canopy height, creating a more even community with a more symmetrical competition to light availability (Ballare *et al.,* 1994).

In a pot experiment with competing species pairs, no relationship between the pot's mean plasticity and community evenness occurred. But when the differences between plasticities in various leaf traits were investigated separately, dissimilarities appeared: when one plant in the pot had higher plasticity in leaf area, leaf number, or leaf length, that species would dominate over the other and create an uneven distribution between the species. Yet the complete opposite happened with plasticity in SLA, where less plastic species dominated over more plastic species (Hermlin, Lepik & Zobel, 2022). In another pot experiment, higher plasticity in SLA reduced pot evenness while higher plasticity in leaf number and leaf length increased it (Hermlin, 2019). It seems that different plastic traits have contrasting effects on species coexistence, as plasticity in SLA tended to have an opposite effect on species coexistence compared to other measured leaf trait plasticities.

Plants can discriminate between different genotypes, making neighbor identity an essential factor for determining the outcome of plant-plant interactions. Morphological plasticity to neighbor identity is significant when the neighbor is a dominant species and interactions with it are frequent (Abakumova, Zobel, Lepik & Semchenko, 2016). Predictability of the environment lowers costs for plastic modifications, so it is favored to be plastic in response to a frequently encountered neighbor. In high water availability, shoot competition between plants exceeds root competition, while in low water availability root competition tends to be higher (Foxx & Fort, 2019). But neighbor identity can play a major role in this. When *Leucanthemum vulgare* and *Potentilla erecta* competed in mesic conditions, the result was highly negative for *P. erecta* with a significant loss of biomass. When grown alone in drought conditions, both were negatively impacted. However, when grown together with con- or heterospecifics, intraspecific facilitation increased *L. vulgare*'s biomass and doubled *P. erecta*'s biomass. But interspecific facilitation only occurred for *L. vulgare* with a 50% rise in biomass, leaving *P. erecta* unaffected (Wang & Callaway, 2021). *Vicia faba* can mobilize phosphorous that is unavailable to *Zea mays*. When two species are grown together, *Z. mays* overlaps its roots more with *V. faba* than grown with conspecifics to gain access to freely moving phosphorous. The species-specific facilitation of *V. faba* through nutrient uptake increases maize's growth and root proliferation compared to maize monoculture (Zhang *et al*., 2020).

On the other hand, plastic reactions that contribute to intraspecific variation can reduce conspecific competition. This is consistent with a clonal invasive plant, *Spartina alterniflora* gaining a considerable advantage from plasticity to light availability in eutrophic conditions (unlimited nitrogen). Different morphotypes vary in shoot height, with shorter ramets being able to increase their light-use efficiency, photosynthetic rate, and SLA under limiting light conditions. This way, *S. alterniflora* can escape high intraspecific competition while still maintaining extreme productivity (Xu *et al*., 2022). A stable coexistence can also be hindered when niche differentiation gives an advantage to one species over the other. Species, that during competition increased their overall nitrogen uptake from the soil and increased their uptake of the most available nitrogen form – ammonium, had higher biomass compared to species that did not change the amount nor the preferred form of nitrogen when competing with others. Plasticity in nitrogen use made some species superior competitors over non-plastic neighbors (Ashton, Miller, Bowman & Suding, 2010).

A significant amount of research has been done concerning plasticity's potential to allow a species to become invasive (Molina-Montenegro, Penuelas, Munné-Bosch & Sardans, 2012). When a plant has higher fitness across different environments than others, it could possess a threat of thriving in many places. A meta-analysis shows an altogether higher plasticity for invasive species compared to non-invasive native species (Davidson, Jennions & Nicotra, 2011). Important traits for contributing to plants' fitness like water and nitrogen use efficiency (Davidson *et al.*, 2011), biomass (Herr-Turoff & Zedler, 2007; Davidson *et* *al.*, 2011), height (Hiatt & Flory, 2020), and root:shoot biomass ratio (Davidson *et al.*, 2011; Hiatt & Flory, 2020), were shown to be significantly more plastic for invasive species. Despite all that, Davidson *et al.*, 2011 found that the high plasticity expressed by invasive species was not as adaptive as expected: with an increase in resources, invasive species did not improve their fitness, nor did they restrain the decline in fitness with a reduction in resources. Native species, on the other hand, were more successful with the decline in resources, indicating a stronger adaption to habitat. Similar results appeared when the plasticity of species having wider niches (generalists) or occupying resource-rich environments compared to specialists was not higher (Dostál, Fischer, Chytrý & Prati, 2017).

It seems that solely plasticity alone does not play a definite role in community assembly but contributes to either stabilizing effects or fitness differences. While it may be tempting to perceive highly plastic plants as possessing greater intelligence, thereby potentially fostering more efficient communities than less plastic plants with evenly distributed shoots and roots, reduced competition, and optimized utilization of shared resources, it is important to recognize that plants, like all organisms, strive to maximize their individual fitness. Consequently, if enhanced plasticity proves advantageous in this regard, the plant will exhibit certain plastic modifications that will influence other members within the community.

## 3.2. Introduction to the 4-year plucking experiment

Since Lepik & Zobel (2015) found a direct link between plasticity and species richness, and Hermlin (2019) and Hermlin, Lepik & Zobel (2022) elaborated on this finding with an understanding, that plasticity in some traits (leaf area and leaf number) have a positive effect on diversity, while plasticity in SLA creates dominants that diminish diversity. For a better understanding of plant morphological plasticity to light availability, we created a permanent plot experiment on Laelatu wooded meadow, where we manipulated quadrat's mean SLA and leaf number plasticity and assessed species richness and abundance. For plasticity manipulations, we plucked out specific plant species with known plasticity indexes to light availability (Hermlin, Lepik & Zobel, 2022), so that mean quadrat plasticity would increase or decrease. The manipulations were carried out twice a year, from June 2019 to September 2022. Our two hypotheses were: 1) greater mean quadrat's plasticity in leaf number has a positive effect on species richness; 2) greater mean quadrat's plasticity in SLA has a negative effect on species richness.

# 4. Material and methods

## 4.1. Study site

Laelatu wooded meadow is located on the cost of western Estonia (58° 35′ N, 23° 33′ E). After the last glacial period (Weichselian glaciation) in about 11 000 years ago, the land started rising and new areas would emerge from the sea. It was not until 2000 years ago when Laelatu would start rising above sea level and begin growing vegetation. Laelatu wooded meadow has been consistently mowed for at least the past 300 years, preventing the establishment of a mature forest ecosystem. Constant management (cutting in July with biomass removal) in addition to a sparse tree canopy cover is inherent for this area and helps to sustain high species richness (Kull & Zobel, 1991; Aavik, Jõgar, Liira, Tulva & Zobel, 2008).

Measured between 1991–2020, the mean temperature in Virtsu (the nearest town to Laelatu, 3,7 km away) for July is 18,2 °C and for January -2,3 °C. Maximum and minimum temperatures for July and January respectively are 32,5 °C and -31,2 °C. The mean annual precipitation is 624 mm. The soil humus layer is thin (20–25 cm) and prone to drying out in summer, but calcareous (pH ≈ 7), as right underneath lies limestone bedrock (soil type: rendzic leptosol) (Kull & Zobel, 1991). Laelatu wooded meadow encompasses an area of 153 ha, yet currently, only approximately 35 ha is mown regularly (Aavik *et al.*, 2008). Vegetation is classified as a *Sesleria caerulea-Filipendula vulgaris* community and the most frequent tree species are *Quercus robur*, *Fraxinus excelsior*, *Betula* spp. and *Populus tremula* (Kull & Zobel, 1991). Laelatu meadow is known for its high small-scale species richness – in 1991 Kull & Zobel counted a maximum of 25 species on a 100 $cm^2$ plot and 42 species on a 400 $cm^2$ plot. In 2001 a record of small-scale species richness for Estonia and Northern Europe was found – 76 species growing on a 1 $m^2$ plot (Kukk, 2004).

## 4.2. Methods

On the 28th of June 2019, the Evolutionary Ecology workgroup of the University of Tartu created permanent plots consisting of 25 quadrats (30x30 cm) on the Laelatu wooded meadow. All plots were placed semi-randomly, planned only so that some would have higher and some lower tree canopy cover. Plots were randomly divided between 5 treatments:

manipulations (2–5) and control (1) (Table 1). In total, there are five plots assigned to each treatment.

Table 1. Treatments

| Treatment nr | Abbreviation | Context |
|---|---|---|
| 1 | C | Control |
| 2 | SLA+ | Increasing the plot's mean SLA plasticity |
| 3 | SLA- | Decreasing the plot's mean SLA plasticity |
| 4 | LN+ | Increasing the plot's mean leaf number plasticity |
| 5 | LN- | Decreasing the plot's mean leaf number plasticity |

All manipulations were carried out by plucking out the aboveground biomass of species with known plasticity. We plucked out species with low plasticity in SLA or leaf number to increase mean plot plasticity (treatments SLA+ and LN+). The same logic relies on SLA- and LN- manipulations: plants with high SLA plasticity had to be removed to decrease plot mean SLA plasticity and plants with high leaf number plasticity had to be removed to decrease plot mean leaf number plasticity. Out of the 105 species found in our plots, the plasticity of various traits to light availability was known for 26 species. However, within these 26 species were some of the most common ones (Table 2). For an example, plants that had the highest plasticity in SLA were *Briza media*, *Prunella vulgaris*, and *Serratula tinctoria*. Plants with the lowest plasticity in SLA were *Festuca rubra*, *Succisa pratensis*, and *Leontodon hispidus*. The most plastic plants in leaf number were *Anthoxanthum odoratum*, *Dactylis glomerata*, and *Plantago lanceolata*, while the least plastic ones were *Carex flacca*, *Ranunculus acris*, and *Serratula tinctoria*. The whole species list and the species list of plucked plants and their corresponding plasticities can be found in Supplementary material S1 and S2.

We carried out the manipulations twice a year – during the beginning of summer and the end of summer – for four years. Specific dates were: 28.06 and 23.08 in 2019, 09.06 and 12.09 in 2020, 07.06 and 17.09 in 2021, and 14.06 and 01.09 in 2022. Before every plucking session, we composed a species list for each plot. Species' coverage was measured once a year, during the first session of the year. We dried the plucked species' above-ground biomass for 48 hours at 75 degrees C and measured the dry weight. Only a species list and species springtime coverage were noted for the control plots.

Table 2. Species that were most common or had the highest coverage.

| Most common | Highest coverage |
|---|---|
| *Carex tomentosa* | *Serratula tinctoria* |
| *Brachypodium pinnatum* | *Pimpinella major* |
| *Biza media* | *Brachypodium pinnatum* |
| *Festuca rubra* | *Convallaria majalis* |
| *Potentilla erecta* | *Potentilla erecta* |
| *Plantago lanceolata* | *Anemone nemoralis* |
| *Prunella vulgaris* | *Sesleria caerulea* |
| *Helictotrichon pratense* | *Prunella vulgaris* |
| *Sesleria caerulea* | *Briza media* |
| *Dactylis glomerata* | *Carex tomentosa* |

*Underlined species are with known plasticity indexes

In 2020, during the second year of the experiment, we incorporated a buffer zone – a 10 cm wide area around the plot from where we plucked the species according to the treatment. The purpose of the buffer zone was to limit unwanted species' opportunities to spread back to the plot quickly. Species plucked from the buffer zone were not collected.

Laelatu wooded meadow is mown annually in July. We mowed the biomass from our plots with scissors to replicate the natural conditions and collect additional information. We used the regular mowing height at the local site (7 cm from the soil level) and collected plot biomass, followed by drying and weighing as written above.

## 4.3. Statistical analyses

Species plasticity of SLA and leaf area were previously calculated as a reaction norm slope between light availability and trait value. For the plasticity estimate to be size-independent and comparable between different species, trait and light availability values were log-transformed, and the allometric effect of biomass was removed. For a more detailed methodology, see Lepik *et al.* (2005).

The plasticity values between SLA and leaf number are different regarding the scale: SLA plasticity values are mostly negative and range from -10,31 to 6,00 but leaf number plasticity values are for the most part positive and range from -2,95 to 7,74. We interpreted that the absolute value of plasticity will give us information about the strength of plasticity, but the direction is meaningless (Dostál *et al.*, 2017). Therefore, we multiplied all the negative SLA plasticity values with -1 for data analysis.

Our experimental technique (removing species) is in contradiction to our hypothesis, that in two manipulations, species richness should grow. To take the effect into account, I calculated a few indicators for our data:

1. Species pool = a constant, that represents all the species found from our plots within four years.
2. Forbidden species = an indicator for each manipulation. It is a constant that represents the number of species "forbidden" in the manipulation. These are the same species we try to remove each year if they appear.
3. Manipulated species pool = Species pool/forbidden species, a constant for each manipulation for stating the number of species that are allowed in this manipulation.
4. Cumulative species richness = number of species noticed within one manipulation throughout four years.
5. Species richness in manipulation = the number of species found in one manipulation, can consist of forbidden species if they were found.
6. Quadrat's mean species richness = sum (species richness in each quadrat within the manipulation)/the number of quadrats within a manipulation (5)
7. Relative richness = Species richness in manipulation/Manipulated species pool
8. Quadrat's relative richness = Quadrat's mean species richness/Manipulated species pool

For making bar plots I multiplied relative richness values by 100 so they would be visually understandable. Also, when looking into the first year of our experiment, to get relative richness values, species richness must be divided by the whole species pool constant, because we identified all species in the plots, whether they became "forbidden" next time or not. For control, as well, relative richness is always calculated by dividing species richness by the whole species pool number.

We used General Linear Model (GLM) with full-factorial ANOVA to describe the relationship between species richness, coverage, biomass, time and manipulations. We used relative richness as an indicator of species richness because it takes into account the “forbidden” species. However, relative richness is a deviation with values ranging from 0 to 1. To bring this deviation of numbers closer to normal deviation, we used the arcsin function of relative richness. We saw time as a categorical variable because in our dataset species richness is observed in specific time points, not on a continuous scale. For continuous variables, in different models we used quadrat coverage or quadrat biomass. We also have biomass and coverage from species that we removed from quadrats, but because they correlated with the whole quadrat biomass and coverage, we are not using them in data analysis.

Species turnover in grasslands can be rather high. With a fast migration rate, there might be a difference in accumulative species richness between manipulations. To further investigate the effect, I calculated cumulative species richness values for all 25 quadrats through 2019–2022, and the growth in species richness from the beginning of the experiment towards the end. These numbers follow the Poisson distribution and are therefore squared to achieve a normal distribution. In addition to the accumulative richness in quadrats, I calculated an overall accumulative richness for all five manipulations. To test if there are any statistically significant differences in accumulative species richness between manipulations, we used a chi-squared test.

All data analyses were done in RStudio (version R4.2.2) and in STATISTICA 7 (StatSoft, Tulsa, OK, USA).

# 5. Results

In together we found 105 species from our plots during this four-year sampling period. At the end of our experiment in June 2022, there was no significant difference in species richness or relative richness between different manipulations (Fig. 1). The manipulation did not have an effect on cumulative species richness either ($x^2 = 1,41$; df = 4; $0,90 > p > 0,75$).

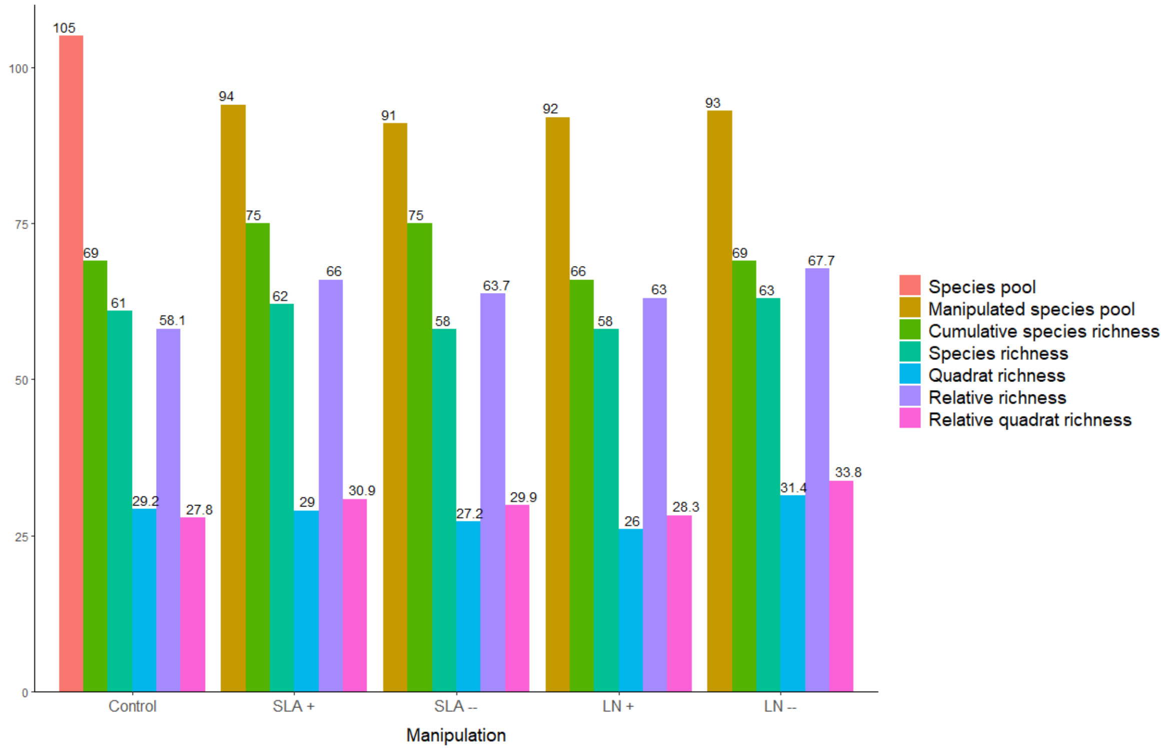

Figure 1. Differences in species pool, cumulative species richness, species richness and relative richness between manipulations at the end of the experiment (June 2022).

Still, removing specific species from quadrats under manipulation showed our success changing quadrat's mean plasticity in the right direction (Fig. 2; Fig. 3). By the fourth year, coverage-weighted mean leaf number plasticity was significantly higher in quadrats with LN + manipulations and lower in LN – manipulations ($p \approx 0,0024$), compared with the first year (Fig. 2). Significant change in quadrat's coverage-weighted mean SLA plasticity also occurred in SLA manipulations, where plucking out highly plastic plants in SLA did lower quadrat's mean SLA plasticity and removing plants with low SLA plasticity on the contrary raised the quadrat's mean SLA plasticity ($p \approx 0,026$) (Fig. 3).

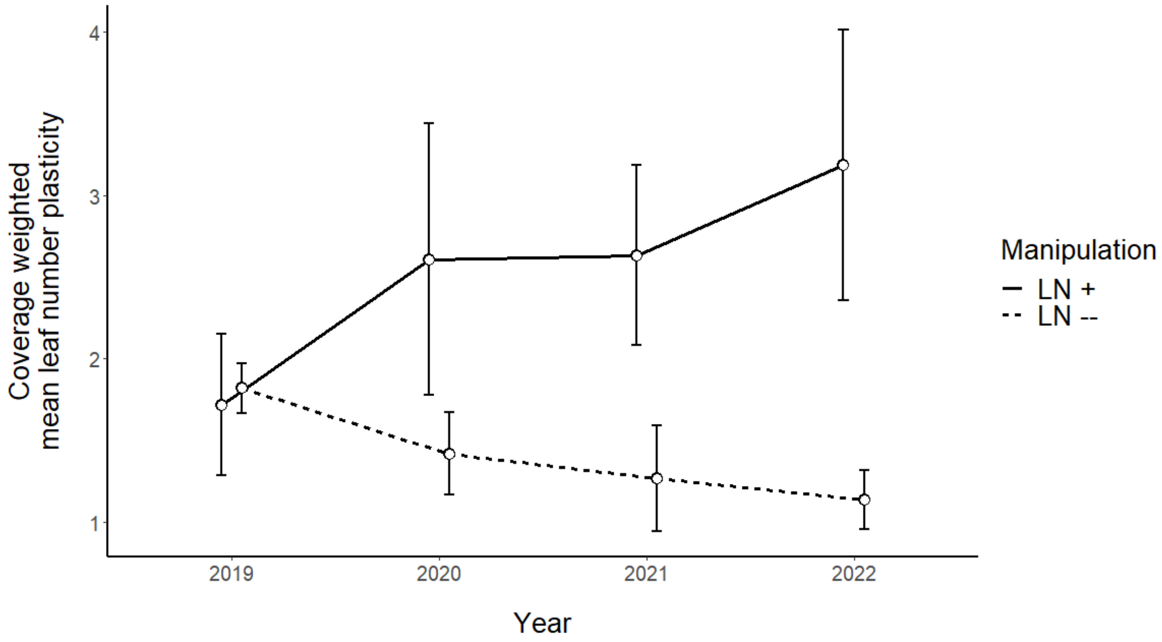

Figure 2. LN+ and LN- manipulation effect on leaf number plasticity from 2019 to 2022. Y-axis represents the weighed mean leaf number plasticity and x-axis represents the years we performed plucking. Manipulation is included as a second factor; 95% confidence intervals are shown. Year*manipulation interaction is significant (p = 0.002444).

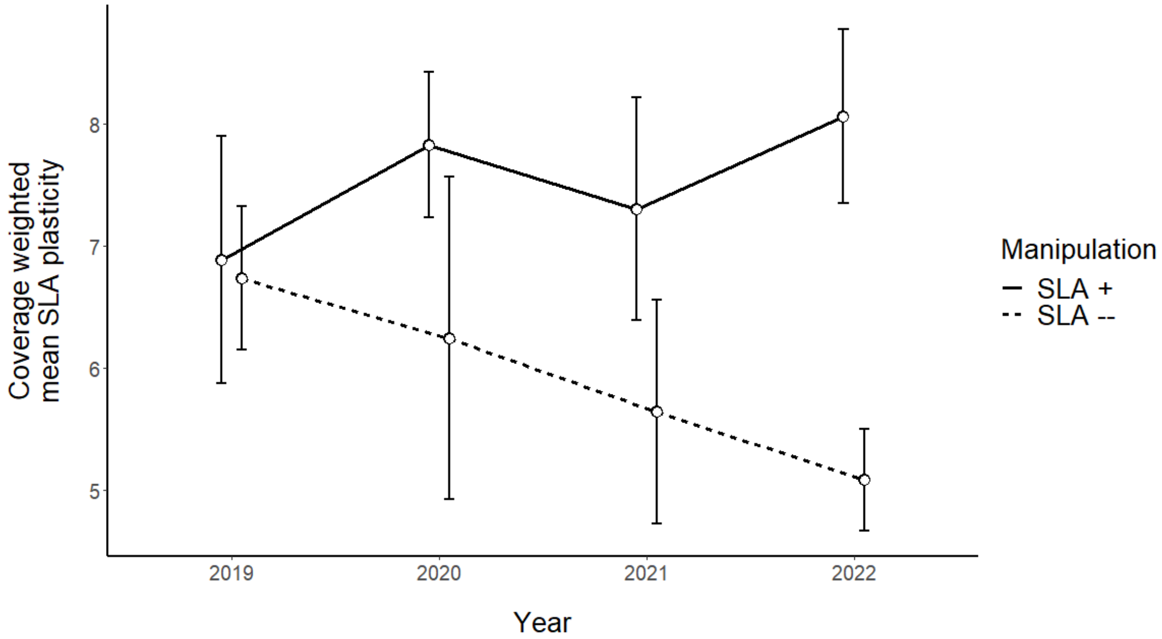

Figure 3. SLA+ and SLA- manipulation effect on specific leaf area (SLA) plasticity from 2019 to 2022. Y-axis represents the weighed mean SLA plasticity and x-axis represents the years we performed plucking. Manipulation is included as a second factor; 95% confidence intervals are shown. Year*manipulation interaction is significant (p = 0.002444).

We used two models to find out the influence our manipulations had on species richness. First, we incorporated quadrat biomass as a continuous variable and discovered a significant interaction between manipulation and quadrat biomass on species richness ($p \approx 0{,}0043$)

(Table 3.; Fig. 4). Also, manipulation had a main effect when biomass was added to the model (p ≈ 0,022).

Table 3. Full-factorial ANOVA table with arcsin (relative richness) being the dependent variable, year and manipulation categorical factors, and biomass as a continuous factor.

| | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Intercept | 0,63 | 1 | 0,63 | 493,06 | < 0,0001 |
| Year | 0,0040 | 3 | 0,0013 | 1,03 | 0,38 |
| **Manipulation** | **0,016** | **4** | **0,0039** | **3,06** | **0,022** |
| Biomass | 0,0016 | 1 | 0,0016 | 1,26 | 0,27 |
| Year*Manipulation | 0,0067 | 12 | 0,00056 | 0,44 | 0,94 |
| Year*Biomass | 0,00069 | 3 | 0,00023 | 0,18 | 0,91 |
| **Manipulation*Biomass** | **0,021** | **4** | **0,0054** | **4,17** | **0,0043** |
| Error | 0,092 | 72 | 0,0013 | | |

Significant results are in bold.

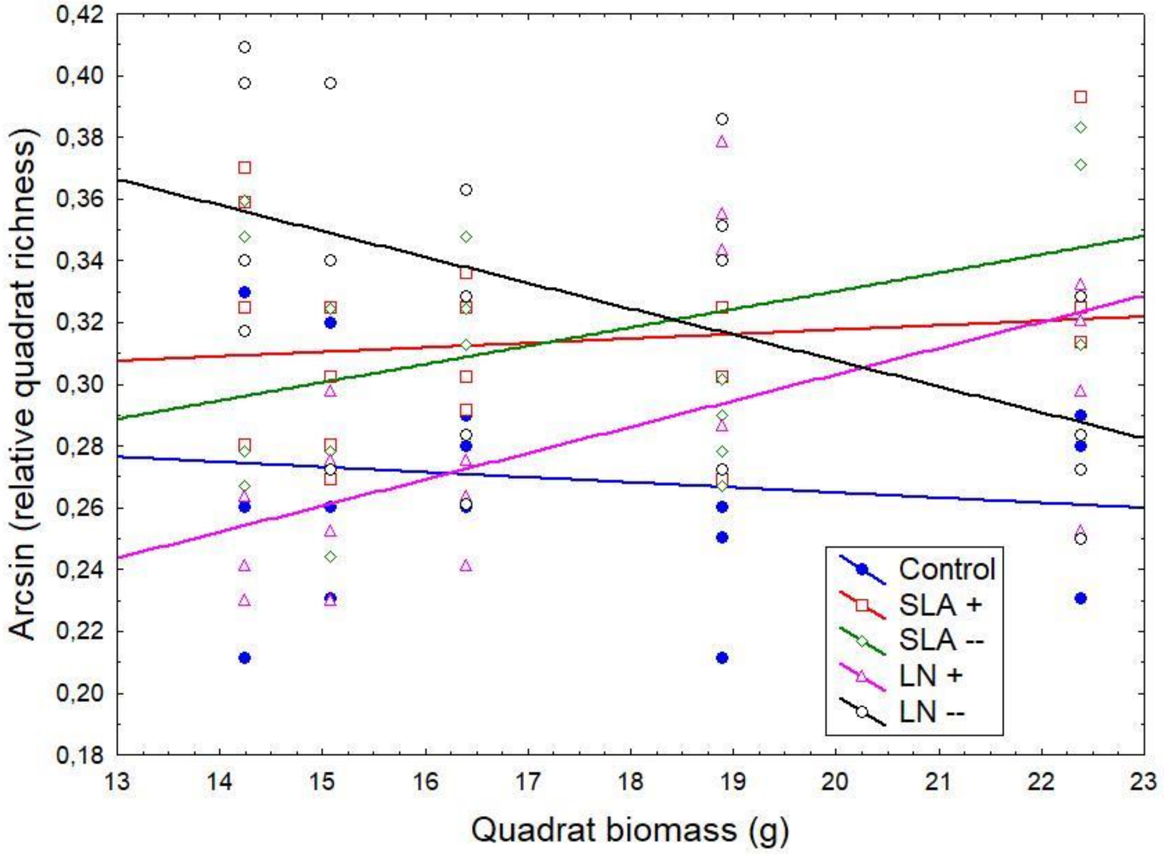

Figure 4. Interaction between quadrat biomass and manipulation in relation to relative species richness. X-axis represents quadrat biomass (g) and y-axis arcsin function of relative richness. Significant (p ≈ 0,0043). Filled dots represent the control.

Figure 4. shows that in general, community biomass has a contrasting effect on species richness regarding different manipulations. Interestingly, if we separated the interaction between manipulation and biomass by manipulation, only the correlation coefficients for LN+ and LN– were significant (pink and black line on Fig. 4) with r- and p-values respectively r = 0,58; p = 0,0074 and r = -0,49; p = 0,028. In LN+ biomass had a strong positive effect on species richness, while for LN– it came out the opposite.

In our second model, we selected quadrat coverage as a continuous variable. Biomass and coverage were related to each other (r = 0,22), but one could describe only 5% of the other. That is why both variables were used in the data analyzing process, but not at the same time in the same model. Coverage had a strong effect on species richness (p < 0,001) (Table 4.; Fig. 5). Eek & Zobel (2001) found similar results in their 5-year community experiment. But manipulation separately or in interaction with year or coverage did not show any significant results regarding species richness.

Table 4. Full-factorial ANOVA table with arcsin (relative richness) being the dependent variable, year and manipulation categorical factors, and coverage a continuous factor.

| | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Intercept | 0,24 | 1 | 0,24 | 211,08 | < 0,0001 |
| Year | 0,0085 | 3 | 0,0028 | 2,49 | 0,067 |
| Manipulation | 0,0026 | 4 | 0,00065 | 0,57 | 0,69 |
| **Coverage** | **0,015** | **1** | **0,015** | **12,92** | **0,00059** |
| Year*Manipulation | 0,0083 | 12 | 0,00070 | 0,62 | 0,82 |
| **Year*Coverage** | **0,014** | **3** | **0,0046** | **4,08** | **0,0099** |
| Manipulation*Coverage | 0,0053 | 4 | 0,0013 | 1,18 | 0,33 |
| Error | 0,082 | 72 | 0,0011 | | |

In bold are significant results

Year and manipulation interaction did not show significant results (p ≈ 0,82) (Fig. 6). Interaction between manipulation and coverage was also non-significant (p ≈ 0,33), but the effect of quadrat coverage on species richness was surprisingly similar for both SLA manipulations (SLA+ : r = -0,11; p = 0,64, SLA- : r = -0,10; p = 0,68). In both SLA manipulations increasing coverage had a weak negative effect on species richness, while other manipulations had weak positive effects.

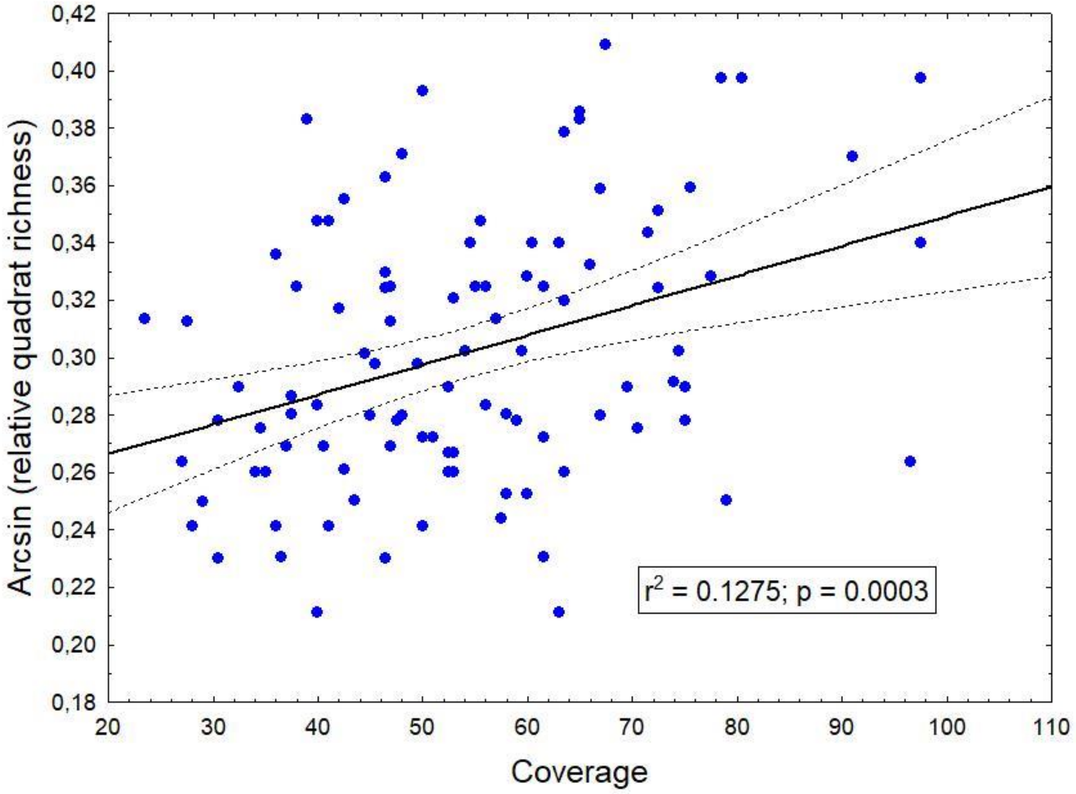

Figure 5. Coverage's effect on relative species richness. Quadrat's coverage is on x-axes and arcsin function of relative richness on y-axes. Data from 4 years is pooled. Significant ($p < 0,001$).

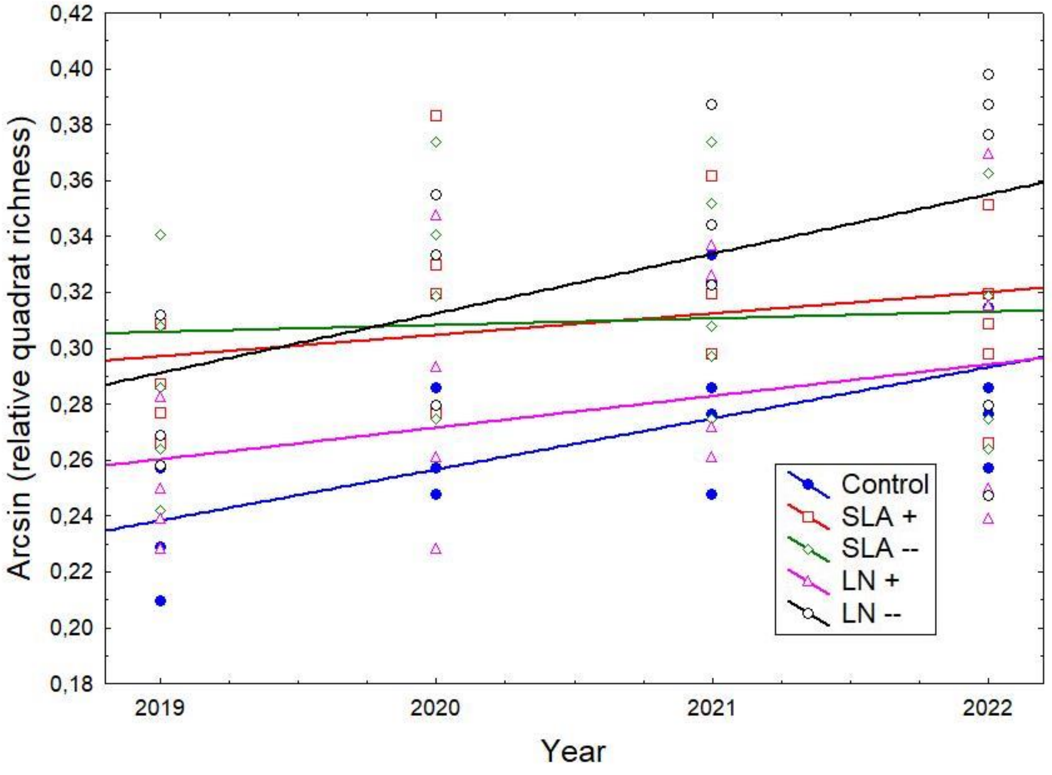

Figure 6. Interaction between year and manipulation in relation to relative species richness. X-axis has categorically years and y-axis arcsin function or relative species richness. Not significant ($p \approx 0,82$).

# 6. Discussion

Our two hypotheses, 1) greater mean plasticity in leaf number has a positive effect on species richness; 2) greater mean plasticity in SLA has a negative effect on species richness, did not find proof. SLA– and LN+ manipulations do not show higher species richness, cumulative richness, or relative richness than SLA+ and LN– manipulations (Fig. 1). Still, when biomass was added to the model, manipulation held a main effect on species richness ($p \approx 0{,}022$), meaning that different manipulations posed an effect on species richness when the effect of biomass was considered. In fact, both SLA manipulations expressed similar results, like in the figure 4 in interaction with biomass, in the figure 6 in interaction with year and in interaction with coverage (SLA+ : $r = -0{,}11$; $p = 0{,}64$, SLA– : $r = -0{,}10$; $p = 0{,}68$), while LN manipulations showed contrasting results, like in figure 4 in interaction with biomass (LN+ : $r = 0{,}58$; $p = 0{,}0074$, LN– : $r = -0{,}49$; $p = 0{,}028$).

While SLA interactions are all non-significant, meaning, they overall do not show an effect on species richness, plasticity in leaf number interacted significantly with quadrat biomass. Higher quadrat productivity had a negative effect on species richness when the quadrat's mean leaf number plasticity was decreased, and a positive effect when together were growing plants with higher leaf number plasticity. As species richness did not change, greater leaf number plasticity might have helped species grow more biomass and adapt to greater light competition that would occur with higher biomass.

The fact that contrasting SLA manipulations had the same, absence of an effect regarding species richness, might indicate that high plasticity in SLA alone does not give species a competitive advantage. Great plasticity in SLA is important for high-light plants to adapt to shading by increasing SLA (wider and thinner leaves) (Valladares & Niinemets, 2008). Many studies associate SLA with competitive interactions between plants (Hess & Levine, 2022), but Roscher, Schumacher, Schmid & Schulze (2015) found that higher SLA plasticity for plants in species mixtures, on the opposite, affected their performance negatively. The optimal SLA value in nature might be lower than the one found in greenhouse pot experiments, as plants with too high SLA values in shade suffer from herbivore damage (Hiatt & Flory, 2020).

However, we did manage to successfully manipulate quadrat plasticity in the right direction (Fig. 2 and Fig. 3). To elucidate why, despite the fact that we were able to change plasticity values in nature, we still did not see the effect plasticity has on species richness, changes in nature must take a while longer than the time we have given them. We were trying to transform a whole community – aggregate species richness in a fraction of the area (10 quadrats x 0,09 $m^2$) under the whole species pool and minimize species richness in another 10 quadrats. Figure 1 illustrates that the cumulative species richness by the fourth year is quite far from the species number in the species pool, indicating that a substantial number of species are rare and not found in all manipulations.

The rate of species turnover is scale-dependent. In micro-scale, within 2x2 cm quadrats, species turnover can be even 42% (species composition changed in 42% of microplots). 80% of new colonization events within the quadrat happened due to clonal growth (Otsus & Zobel, 2002). Most of the species in our species pool are also clonal, therefore micro-scale movements must be frequent. However, species movement between 30x30 cm quadrats in about 400 $m^2$ land takes longer. Klimeš (1999) detected that species-rich grassland species were less mobile than expected by a null model during his six-year study. Annual plants colonized greater distances more often than clonal plants, which persisted in their location more frequently than expected.

The increase in quadrat coverage with increasing species richness is something observed before. Eek & Zobel (2001) found that positive species richness and coverage relationship held against all treatments (illumination, fertilization, mowing). They expected the species richness to decline in plots with high coverage under fertilization and non-mowing treatments but that did not happen. As Laelatu wooded meadow is under annual mowing, species cannot grow too big and express their dominative abilities and coverage becomes a positive estimate of species richness. But we also found, that in different years, coverage had a different effect on species richness ($p \approx 0{,}0099$), whereas manipulation did not ($p \approx 0{,}33$). Coverage gained a more positive effect on species richness by each year because, with a species removal, we created space that could be occupied by two new species, and thus have an increase in coverage and species richness. But it seems that it did not matter which species we removed.

Our experiment was based on the results of previous plasticity experiments done partly in Laelatu meadow (Lepik & Zobel, 2015) and in greenhouse (Hermlin, 2019 and Hermlin, Lepik & Zobel, 2022), but our results did not come out as we expected based on these previous studies. Hermlin (2019) found a strong negative connection between SLA plasticity and pot evenness, and positive interaction between leaf number plasticity and pot evenness, indicating that greater SLA plasticity gave some species competitive advantages over the others, while greater leaf number plasticity operated the opposite way. It might be the fault of a totally different experimental design that has not allowed our study to express similar results, as discussed in chapter 2.1.

Since Hermlin (2019) used small four-species communities with 40 individuals, the same higher-order interactions, that act as stabilizing mechanisms in nature and enable similar species to coexists, were missing in her study but present in ours. I speculate that these stabilizing interactions might have alleviated the competitive effect species with higher SLA plasticity might have on others and thus allowed for higher species richness even in SLA+ manipulation. When two or four species compete over limited resources, competitive outcomes are usually different than when the competition takes place in nature, surrounded by other species, that control each other's densities, and special heterogeneity. Levine, Bascompte, Adler & Allesina (2017) raise these topics in their review that collects the sparse evidence we have on complicated species interactions. Even if we keep the fundamental principle of all coexistence theory intact, that all interactions between species are fundamentally pairwise, coexistence arises from higher-order interactions. In that way, one species cannot have that great impact on another species, because it itself is being limited by a third species and so one. Most pairwise interactions end with the competitive exclusion of the weaker competitor(s), like in Adler, Ellner & Levine (2010).

*Festuca rubra* is a superior competitor for nutrients to *Plantago lanceolata* when both species are grown in monocultures. *F. rubra* can grow deeper roots and access otherwise limiting nitrates that remain unused for shorter roots of *P. lanceolata*. Considering this information, from two species *F. rubra* should be the winner in interspecific competition. However, in mixtures, *P. lanceolata* suppressed the root growth of *F. rubra* by 72% and increased its own root growth by 252% when compared with monocultures (Padilla *et al.*, 2013). This kind of higher-order interaction by *P. lanceolata* will reduce the interspecific competition between *F. rubra* and a third competitor for limiting nutrients in regard to intraspecific competition and behave as a stabilizing mechanism in the community (Levine *et al.*, 2017).

Community is a complex system and removing different species with different functions can affect aboveground biomass and coverage, as well as belowground communities and soil biota. But species also modify their environment, with the creation of a species-specific soil biota. When graminoid species were removed from the community, it was easier for them to move back in than for other species to take their place. The soil biota might alleviate the colonization of a certain species and inhibit others (Helsen, Hermy & Honnay, 2016). Another study came into conclusion, that soil conditioning is not the most important factor inhibiting colonization of soil patches, but species' own spreading abilities. Clonal plants with high lateral spread were the first ones established in those patches, the next year seedlings with high growth rates appeared. The trade-off between colonization and competition provides a coexistence benefitting mechanism by allowing inferior competitors colonize free space first (in 't Zandt, Hoekstra, de Caluwe, Cruijsen, Visser & de Kroon, 2022).

Our species removal treatment can be compared with grazers, who create gaps in the community. The effect on diversity depends on the species getting eaten. When a dominant species is negatively affected by grazing, species that were suppressed by this dominant gain an advantage (Liang, Gornish, Mariotte, Chen & Liang, 2019). Grazing can have a positive effect on species richness with higher species turnover in grazed sites compared to ungrazed sites. Also, gap size is important, with small gaps (3 cm diameter) getting colonized more quickly and densely than bigger ones. But even in the smallest gaps, ramet densities did not recover to the initial pre-disturbance state (Bullock, Hill, Silvertown & Sutton, 1995).

We registered an increase (though non-significant with both models) in species richness during our four-year experiment. Part of it may be explained by the increase in our skills in identifying different plant species over the years. Figure 5 shows a non-significant relationship between the interaction of year and manipulation and their effect on species richness. Interestingly, it might show our learning curve in becoming more advanced with species recognition. In control, where no plucking was done, we can still see an increase in species richness. However, there also were new species, that colonized free spaces, regardless of our identifying skills. Partly this increase in species richness can be our contribution to spreading species while walking between plots and helping them colonize new areas (Otsus & Zobel, 2002). It is logical that the observed species richness increased with the first years as some plants are dormant and do not show themselves each year, besides, the natural flow of species that immigrate our plots or are lost from them is around 10%. For this effect to dissolve, we need more time to find as many species as possible from our area. As species move around in the community, it is possible, that at one point, most of them have reached all parts of the area (van der Maarel & Sykes, 1993).

# Conclusions

Understanding the environment–community assembly relationship is a challenge. With so many variables shifting the outcome of species interactions in one or the other direction. Those variables can benefit species coexistence by creating stabilizing niche differences between species and heterogeneity in space so that one species would suppress its own growth more than its heterospecific neighbor's. Or create stronger fitness differences between species that lead to competitive dominance and an impediment to coexistence.

Plasticity, the ability of a genotype to generate a fitting phenotype to the range of environmental conditions, is important for plants for adapting to environmental change. It can do both – differentiate species for stabilizing effects and give competitive advantages to one of them. Still, higher morphological plasticity to light availability is shown to increase species richness through improving the evenness of plant height and lowering asymmetric competition for light.

In this thesis, I analyze, how two types of plasticity – plasticity in leaf number and in specific leaf area (SLA) – will impact species richness based on a four-year species removal experiment in Laelatu wooded meadow. Two hypotheses: 1) greater mean plasticity in leaf number has a positive effect on species richness; 2) greater mean plasticity in SLA has a negative effect on species richness, do not find proof. There probably has not been enough time for changes in the community to become evident. There is a strong positive relationship between plot coverage and species richness as well as a biomass mediated effect of different species removal on species richness. But one day the subtle changes in community dynamics will add up and hopefully a pattern emerges. Until then I must carry on the experiment.

# Kokkuvõte

**Taimede morfoloogilise plastilisuse mõju taimekoosluse mitmekesisusele: nelja-aastane püsiruudukatse Laelatu puisniidul.**

Keskkonna täpset mõju kooslusele on raske hinnata. Nii paljud eri tegurid võivad täiesti isesugustes suundades kujundada liikidevahelisi suhteid. Parendada liikide kooseksisteerimist, luues liikide vahele stabiliseerivaid nišierisusi ja varieeruvust ruumis, et liik suruks enda kasvu rohkem maha kui oma teistliiki naabri oma. Või vastupidiselt läbi suurenenud fitnessi erinevuste anda ühele liigile konkurentsis eelis teise üle ja pärssida kooseksisteerimist.

Plastilisus, genotüübi võime luua erinevates keskkondades just sinna sobiv fenotüüp, on taimedele oluline oskus keskkonnamuutustega kohanemisel. Plastilisus võib olla võtmetegur mõlemas olukorras – muuta liikide tunnuseid üksteisest erinevamaks stabiliseeriva efekti ilmumise jaoks ja ka anda konkurentsis eelis ühele liigile teise üle. Siiski, on näidatud, et kõrgem morfoloogiline plastilisus valguse kättesaadavusele võib suurendada koosluse liigirikkust läbi ühtlasema pikkusega võsude loomise ja seeläbi ebasümmeetrilise valguskonkurentsi vähendamise.

Selles magistritöös ma analüüsin, kuidas plastilisus lehtede arvus ja lehe eripinnas (SLA) mõjutab liigirikkust nelja-aastase Laelatu puisniidu kitkumiskatse põhjal. Töö kaks hüpoteesi – 1) suurem SLA plastilisus vähendab koosluse liigirikkust; 2) suurem lehtede arvu plastilisus suurendab koosluse liigirikkust – ei leia tõestust. Arvatavasti pole kõik väiksed muutused koosluses veel avalduda jõudnud. Oluline positiivne seos ilmneb ruudu katvuse ja liigirikkuse vahel ning koosmõjus ruudu biomassi ja liikide eemaldamise vahel liigirikkusele. Seega, on tarvis eksperimendiga jätkata, et muutuste tekkele rohkem aega anda ning loota kunagi sealt selgema mustri avaldumisele.

# Acknowledgments

I am sincerely thankful to my supervisor, Hanna Kaarin Hermlin, for supporting and encouraging me through these years. She is the absolute best companion for going on sampling trips to Laelatu wooded meadow, which makes me slightly hope our experiment will continue to last. I also want to thank Kristjan Zobel for guiding me through the data analyzing process and teaching me how to see the world through Poisson distribution.

# References

Aavik, T., Jõgar, Ü., Liira, J., Tulva, I., & Zobel, M. (2008). Plant diversity in a calcareous wooded meadow–The significance of management continuity. *Journal of Vegetation Science*, *19*(4), 475-484.

Abakumova, M., Zobel, K., Lepik, A., & Semchenko, M. (2016). Plasticity in plant functional traits is shaped by variability in neighbourhood species composition. *New Phytologist*, *211*(2), 455-463.

Adler, P. B., Ellner, S. P., & Levine, J. M. (2010). Coexistence of perennial plants: an embarrassment of niches. *Ecology letters*, *13*(8), 1019-1029.

Adler, P. B., Smull, D., Beard, K. H., Choi, R. T., Furniss, T., Kulmatiski, A., ... & Veblen, K. E. (2018). Competition and coexistence in plant communities: intraspecific competition is stronger than interspecific competition. *Ecology letters*, *21*(9), 1319-1329.

Aguiar, M. R., Lauenroth, W. K., & Peters, D. P. (2001). Intensity of intra-and interspecific competition in coexisting shortgrass species. *Journal of Ecology*, *89*(1), 40-47.

Arnold, P. A., Nicotra, A. B., & Kruuk, L. E. (2019). Sparse evidence for selection on phenotypic plasticity in response to temperature. *Philosophical Transactions of the Royal Society B*, *374*(1768), 20180185.

Ashton, I. W., Miller, A. E., Bowman, W. D., & Suding, K. N. (2010). Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. *Ecology*, *91*(11), 3252-3260.

Ballaré, C. L., Scopel, A. L., Jordan, E. T., & Vierstra, R. D. (1994). Signaling among neighboring plants and the development of size inequalities in plant populations. Proceedings of the National Academy of Sciences, 91(21), 10094-10098.

Barot, S., & Gignoux, J. (2004). Mechanisms promoting plant coexistence: can all the proposed processes be reconciled?. *Oikos*, *106*(1), 185-192.

Bennett, J. A., & Klironomos, J. (2019). Mechanisms of plant–soil feedback: interactions among biotic and abiotic drivers. *New Phytologist*, *222*(1), 91-96.

Bertness, M. D., & Callaway, R. (1994). Positive interactions in communities. *Trends in ecology & evolution*, *9*(5), 191-193.

Bonser, S. P. (2021). Misinterpreting the adaptive value of phenotypic plasticity in studies on plant adaptation to new and variable environments. *Plant Biology*, *23*(5), 683-685.

Bradshaw, A. D. (1965). Evolutionary significance of phenotypic plasticity in plants. *Advances in genetics*, *13*, 115-155.

Brooker, R., Brown, L. K., George, T. S., Pakeman, R. J., Palmer, S., Ramsay, L., ... & Wilkinson, M. J. (2022). Active and adaptive plasticity in a changing climate. *Trends in Plant Science*.

Burns, J. H., & Strauss, S. Y. (2012). Effects of competition on phylogenetic signal and phenotypic plasticity in plant functional traits. *Ecology*, *93*(sp8), S126-S137.

Cai, Q., Zhang, Y., Sun, Z., Zheng, J., Bai, W., Zhang, Y., ... & Zhang, L. (2017). Morphological plasticity of root growth under mild water stress increases water use efficiency without reducing yield in maize. *Biogeosciences*, *14*(16), 3851-3858.

Callaway, R. M. (2007). *Positive interactions and interdependence in plant communities*. Springer Science & Business Media.

Callaway, R. M., Nadkarni, N. M., & Mahall, B. E. (1991). Facilitation and interference of Quercus douglasii on understory productivity in central California. *Ecology*, *72*(4), 1484-1499.

Callaway, R. M., Pennings, S. C., & Richards, C. L. (2003). Phenotypic plasticity and interactions among plants. *Ecology*, *84*(5), 1115-1128.

Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual review of Ecology and Systematics*, *31*(1), 343-366.

Chu, C. J., Maestre, F. T., Xiao, S., Weiner, J., Wang, Y. S., Duan, Z. H., & Wang, G. (2008). Balance between facilitation and resource competition determines biomass–density relationships in plant populations. *Ecology letters*, *11*(11), 1189-1197.

Davidson, A. M., Jennions, M., & Nicotra, A. B. (2011). Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology letters*, *14*(4), 419-431.

De Kroon, H., Huber, H., Stuefer, J. F., & Van Groenendael, J. M. (2005). A modular concept of phenotypic plasticity in plants. *New phytologist*, *166*(1), 73-82.

DeWitt, T. J., Sih, A., & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends in ecology & evolution*, *13*(2), 77-81.

Dostál, P., Fischer, M., Chytrý, M., & Prati, D. (2017). No evidence for larger leaf trait plasticity in ecological generalists compared to specialists. *Journal of Biogeography*, *44*(3), 511-521.

Dudley, S. A., & Schmitt, J. (1996). Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in Impatiens capensis. *The American Naturalist*, 147(3), 445-465.

Ehlers, B. K., & Bilde, T. (2019). Inclusive fitness, asymmetric competition and kin selection in plants. *Oikos*, *128*(6), 765-774.

Fajardo, A., & McIntire, E. J. (2011). Under strong niche overlap conspecifics do not compete but help each other to survive: facilitation at the intraspecific level. *Journal of Ecology*, *99*(2), 642-650.

Fernández-Milmanda, G. L., & Ballaré, C. L. (2021). Shade avoidance: expanding the color and hormone palette. *Trends in plant science*, *26*(5), 509-523.

Foxx, A. J., & Fort, F. (2019). Root and shoot competition lead to contrasting competitive outcomes under water stress: A systematic review and meta-analysis. *PloS one*, *14*(12), e0220674.

García-Cervigón, A. I., Gazol, A., Sanz, V., Camarero, J. J., & Olano, J. M. (2013). Intraspecific competition replaces interspecific facilitation as abiotic stress decreases: The shifting nature of plant–plant interactions. *Perspectives in Plant Ecology, Evolution and Systematics*, *15*(4), 226-236.

Gilroy, S., & Trewavas, A. (2001). Signal processing and transduction in plant cells: the end of the beginning?. *Nature Reviews Molecular Cell Biology*, *2*(4), 307-314.

Goldenheim, W. M., Irving, A. D., & Bertness, M. D. (2008). Switching from negative to positive density-dependence among populations of a cobble beach plant. *Oecologia*, *158*, 473-483.

Govaert, L., Gilarranz, L. J., & Altermatt, F. (2021). Competition alters species' plastic and genetic response to environmental change. *Scientific Reports*, *11*(1), 23518.

Gross, K. (2008). Positive interactions among competitors can produce species-rich communities. *Ecology letters*, *11*(9), 929-936.

Götzenberger, L., de Bello, F., Bråthen, K. A., Davison, J., Dubuis, A., Guisan, A., ... & Zobel, M. (2012). Ecological assembly rules in plant communities—approaches, patterns and prospects. *Biological reviews*, *87*(1), 111-127.

Hamilton, W. D. (1964). The genetical evolution of social behaviour. II. *Journal of theoretical biology*, *7*(1), 17-52.

Helsen, K., Hermy, M., & Honnay, O. (2016). A test of priority effect persistence in semi-natural grasslands through the removal of plant functional groups during community assembly. *BMC ecology*, *16*, 1-11.

Hermlin, H K. (2019). The role of morphological plasticity in plant species coexistence. Magistritöö. Tartu Ülikool, botaanika osakond.

Hermlin, H. K., & Zobel, K. Measuring root plasticity to nutrient concentration and heterogeneity of 16 herbaceous species. *Unpublished.*

Hermlin, H. K., Lepik, M., & Zobel, K. (2022). The importance of shoot morphological plasticity on plant coexistence: a pot experiment. *Plant Biology*, *24*(5), 791-797.

Herr-Turoff, A., & Zedler, J. B. (2007). Does morphological plasticity of the Phalaris arundinacea canopy increase invasiveness?. *Plant Ecology*, *193*, 265-277.

Hess, C., Levine, J. M., Turcotte, M. M., & Hart, S. P. (2022). Phenotypic plasticity promotes species coexistence. *Nature ecology & evolution*, *6*(9), 1256-1261.

Hiatt, D., & Flory, S. L. (2020). Populations of a widespread invader and co-occurring native species vary in phenotypic plasticity. *New Phytologist*, *225*(1), 584-594.

Hutchinson, G. E. (1959). Homage to Santa Rosalia or why are there so many kinds of animals?. *The American Naturalist*, *93*(870), 145-159.

Jung, V., Violle, C., Mondy, C., Hoffmann, L., & Muller, S. (2010). Intraspecific variability and trait-based community assembly. *Journal of ecology*, *98*(5), 1134-1140.

Klimeš, L. (1999). Small-scale plant mobility in a species-rich grassland. *Journal of Vegetation Science*, *10*(2), 209-218.

Kraft, N. J., Godoy, O., & Levine, J. M. (2015). Plant functional traits and the multidimensional nature of species coexistence. *Proceedings of the National Academy of Sciences*, *112*(3), 797-802.

Kukk, T. (2004). Pärandkooslused: kas pärandame nad tulevikule? *Eesti Loodus*, *7*, 6–11. (in Estonian).

Kull, K., & Zobel, M. (1991). High species richness in an Estonian wooded meadow. *Journal of vegetation science*, *2*(5), 715-718.

LaManna, J. A., Mangan, S. A., Alonso, A., Bourg, N. A., Brockelman, W. Y., Bunyavejchewin, S., ... & Myers, J. A. (2017). Plant diversity increases with the strength of negative density dependence at the global scale. *Science*, *356*(6345), 1389-1392.

Leger, E. A., & Espeland, E. K. (2010). The shifting balance of facilitation and competition affects the outcome of intra-and interspecific interactions over the life history of California grassland annuals. *Plant Ecology, 208*, 333-345.

Lepik, M., & Zobel, K. (2015). Is the positive relationship between species richness and shoot morphological plasticity mediated by ramet density or is there a direct link?. *Oecologia*, *178*, 867-873.

Lepik, M., Liira, J., & Zobel, K. (2005). High shoot plasticity favours plant coexistence in herbaceous vegetation. *Oecologia*, *145*, 465-474.

Levis, N. A., Fuller, C. G., & Pfennig, D. W. (2020). An experimental investigation of how intraspecific competition and phenotypic plasticity can promote the evolution of novel, complex phenotypes. *Biological Journal of the Linnean Society*, *131*(1), 76-87.

Mack, R. N., & Harper, J. L. (1977). Interference in dune annuals: spatial pattern and neighbourhood effects. *The Journal of Ecology*, 345-363.

Meilhac, J., Deschamps, L., Maire, V., Flajoulot, S., & Litrico, I. (2020). Both selection and plasticity drive niche differentiation in experimental grasslands. *Nature Plants*, *6*(1), 28-33.

Michalet, R., Brooker, R. W., Cavieres, L. A., Kikvidze, Z., Lortie, C. J., Pugnaire, F. I., ... & Callaway, R. M. (2006). Do biotic interactions shape both sides of the humped-back model of species richness in plant communities?. *Ecology letters*, *9*(7), 767-773.

Molina-Montenegro, M. A., Penuelas, J., Munné-Bosch, S., & Sardans, J. (2012). Higher plasticity in ecophysiological traits enhances the performance and invasion success of Taraxacum officinale (dandelion) in alpine environments. *Biological Invasions*, *14*, 21-33.

Ninkovic, V., Rensing, M., Dahlin, I., & Markovic, D. (2019). Who is my neighbor? Volatile cues in plant interactions. *Plant Signaling & Behavior*, *14*(9), 1634993.

Otsus, M., & Zobel, M. (2002). Small-scale turnover in a calcareous grassland, its pattern and components. *Journal of Vegetation Science*, *13*(2), 199-206.

Pérez-Ramos, I. M., Matías, L., Gómez-Aparicio, L., & Godoy, Ó. (2019). Functional traits and phenotypic plasticity modulate species coexistence across contrasting climatic conditions. *Nature communications*, *10*(1), 2555.

Polechová, J., & Storch, D. (2008). Ecological niche. *Encyclopedia of ecology*, *2,* 1088-1097.

Schiffers, K., Tielbörger, K., Tietjen, B., & Jeltsch, F. (2011). Root plasticity buffers competition among plants: theory meets experimental data. *Ecology*, *92*(3), 610-620.

Schneider, H. M. (2022). Characterization, costs, cues and future perspectives of phenotypic plasticity. *Annals of Botany*, *130*(2), 131-148.

Semchenko, M., Lepik, M., Götzenberger, L., & Zobel, K. (2012). Positive effect of shade on plant growth: amelioration of stress or active regulation of growth rate?. *Journal of ecology*, *100*(2), 459-466.

Stotz, G. C., Salgado-Luarte, C., Escobedo, V. M., Valladares, F., & Gianoli, E. (2021). Global trends in phenotypic plasticity of plants. *Ecology Letters*, *24*(10), 2267-2281.

Subrahmaniam, H. J., Roby, D., & Roux, F. (2021). Toward Unifying Evolutionary Ecology and Genomics to Understand Positive Plant–Plant Interactions Within Wild Species. *Frontiers in Plant Science*, *12*, 683373.

Sultan, S. E. (2000). Phenotypic plasticity for plant development, function and life history. *Trends in plant science*, *5*(12), 537-542.

Zhang, D., Lyu, Y., Li, H., Tang, X., Hu, R., Rengel, Z., ... & Shen, J. (2020). Neighbouring plants modify maize root foraging for phosphorus: coupling nutrients and neighbours for improved nutrient-use efficiency. *New Phytologist*, *226*(1), 244-253.

Zobel, M. (1997). The relative of species pools in determining plant species richness: an alternative explanation of species coexistence?. *Trends in ecology & evolution*, *12*(7), 266-269.

Zuppinger-Dingley, D., Schmid, B., Petermann, J. S., Yadav, V., De Deyn, G. B., & Flynn, D. F. (2014). Selection for niche differentiation in plant communities increases biodiversity effects. *Nature, 515*(7525), 108-111.

Takahashi, F., Kuromori, T., Urano, K., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2020). Drought stress responses and resistance in plants: From cellular responses to long-distance intercellular communication. *Frontiers in plant science*, *11*, 1407.

Tilman, D. (1982). *Resource competition and community structure* (No. 17). Princeton university press.

Turcotte, M. M., & Levine, J. M. (2016). Phenotypic plasticity and species coexistence. *Trends in ecology & evolution*, *31*(10), 803-813.

Wang, S., & Callaway, R. M. (2021). Plasticity in response to plant–plant interactions and water availability. *Ecology*, *102*(6), e03361.

West, S. A., Pen, I., & Griffin, A. S. (2002). Cooperation and competition between relatives. *Science*, *296*(5565), 72-75.

Whitfield, J. (2002). Ecology: neutrality versus the niche. *Nature*, 417(6888), 480-482.

Xu, X., Zhou, C., He, Q., Qiu, S., Zhang, Y., Yang, J., ... & Nie, M. (2022). Phenotypic plasticity of light use favors a plant invader in nitrogen-enriched ecosystems. *Ecology*, *103*(5), e3665.

van Gelderen, K., Kang, C., & Pierik, R. (2018). Light signaling, root development, and plasticity. *Plant physiology, 176*(2), 1049-1060.

Roscher, C., Schumacher, J., Schmid, B., & Schulze, E. D. (2015). Contrasting effects of intraspecific trait variation on trait-based niches and performance of legumes in plant mixtures. *PLoS One*, *10*(3), e0119786.

Padilla, F. M., Mommer, L., de Caluwe, H., Smit-Tiekstra, A. E., Wagemaker, C. A., Ouborg, N. J., & de Kroon, H. (2013). Early root overproduction not triggered by nutrients decisive for competitive success belowground. *PLoS One*, *8*(1), e55805.

in ‘t Zandt, D., Hoekstra, N. J., de Caluwe, H., Cruijsen, P. M., Visser, E. J., & de Kroon, H. (2022). Plant life-history traits rather than soil legacies determine colonisation of soil patches in a multi-species grassland. *Journal of Ecology*, *110*(4), 889-901.

van der Maarel, E., & Sykes, M. T. (1993). Small-scale plant species turnover in a limestone grassland: the carousel model and some comments on the niche concept. *Journal of vegetation science*, *4*(2), 179-188.

Liang, M., Gornish, E. S., Mariotte, P., Chen, J., & Liang, C. (2019). Foliar nutrient content mediates grazing effects on species dominance and plant community biomass. *Rangeland Ecology & Management*, *72*(6), 899-906.

Bullock, J. M., Hill, B. C., Silvertown, J., & Sutton, M. (1995). Gap colonization as a source of grassland community change: effects of gap size and grazing on the rate and mode of colonization by different species. *Oikos*, 273-282.

Valladares, F., & Niinemets, Ü. (2008). Shade tolerance, a key plant feature of complex nature and consequences. *Annual Review of Ecology, Evolution, and Systematics*, *39*, 237-257.

Bakker, C., Blair, J. M., & Knapp, A. K. (2003). Does resource availability, resource heterogeneity or species turnover mediate changes in plant species richness in grazed grasslands?. *Oecologia*, *137*, 385-391.

# Additional materials

Table 1. Species plasticity indexes of leaf number and SLA.

| **Species** | **Leaf number plasticity** | **SLA plasticity** |
|---|---|---|
| *Agrostis stolonifera* | 5,77 | -7,6 |
| *Angelica sylvestris* | 3,18 | -9 |
| *Anthoxanthum odoratum* | 7 | -5,653 |
| *Brachypodium pinnatum* | 1,08 | -5 |
| *Briza media* | 3,17 | -10,31 |
| *Calamagrostis epigejos* | 0,87 | -6,895 |
| *Carex flacca* | 0 | -4 |
| *Centaurea jacea* | 3,28 | -9 |
| *Dactylis glomerata* | 7,648 | -4,422 |
| *Festuca arundinacea* | 0 | -5,66 |
| *Festuca rubra* | 5,046 | -3,1 |
| *Filipendula ulmaria* | 0,11 | 6 |
| *Filipendula vulgaris* | 0,175 | -6,87 |
| *Galium boreale* | -1,8 | -9,121 |
| *Geum rivale* | 4,2 | -7,79 |
| *Leontodon hispidus* | 2,3 | -2 |
| *Molinia caerulea* | -2,95 | -5,42 |
| *Plantago lanceolata* | 7,74 | -8,16 |
| *Plantago media* | 5,3 | -7,6 |
| *Prunella vulgaris* | 0 | -10 |
| *Ranunculus acris* | 0 | -5,57 |
| *Serratula tinctoria* | 0 | -10 |
| *Sesleria caerulea* | 2,85 | -6,072 |
| *Solidago virgaurea* | 0,33 | -6,4 |
| *Succisa pratensis* | 0 | -2,2 |

Table 2. Species list.

**Species**

*Agrostis stolonifera*
*Anthoxanthum odoratum*
*Brachypodium pinnatum*
*Briza media*
*Calamagrostis epigejos*
*Carex capillaris*
*Carex caryophyllea*
*Carex flacca*
*Carex ornithopoda*
*Carex panicea*
*Carex tomentosa*
*Carex vaginata*
*Dactylis glomerata*
*Deschampsia cespitosa*
*Festuca arundinacea*
*Festuca ovina*
*Festuca pratensis*
*Festuca rubra*
*Helictotrichon pratense*
*Helictotrichon pubescens*
*Luzula sp*
*Luzula campestris*
*Luzula multiflora*
*Luzula piloza*
*Molinia caerulea*
*Phleum pratense*
*Sesleria caerulea*
*Aegopodium podagraria*
*Alchemilla vulgaris*
*Allium sp*
*Anemone nemorosa*
*Anemone ranunculoides*
*Angelica sylvestris*
*Asperula tinctoria*
*Betula pendula*
*Campanula glomerata*
*Campanula rotundifolia*
*Campanula persicifolia*
*Centaurea jacea*
*Convallaria majalis*
*Cornus sanguinea*
*Crepis praemorsa*
*Epipactis helleborine*
*Filipendula ulmaria*
*Filipendula vulgaris*
*Frangula alnus*
*Fraxinus excelsior*
*Fragaria vesca*
*Galium album*
*Galium boreale*
*Geum rivale*
*Gymnadenia conopsea*
*Inula salicina*
*Helianthmum nummularium*
*Hepatica nobilis*
*Hieracium umbellatum*
*Hypericum maculatum*
*Hypericum perforatum*
*Lathyrus pratensis*
*Lathyrus cernus*
*Leontodon hispidus*
*Leucanthemum vulgare*
*Linum catharticum*
*Listera ovata*
*Medicago lupulina*
*Melampyrum nemorosum*
*Ophioglossum vulgatum*
*Parnassia palustris*
*Paris quadrifolia*
*Pilosella officinarum*
*Pimpinella major*
*Pimpinella saxifraga*
*Plantago lanceolata*
*Plantago media*
*Polygala amarella*
*Polygonatum odoratum*
*Populus tremula*
*Potentilla erecta*
*Primula veris*
*Prunella vulgaris*
*Pyrola rotundifolia*
*Quercus robur*
*Ranunculus acris*
*Ranunculus polyanthemos*
*Rhamnus cathartica*
*Rhinanthus minor*
*Rubus saxatilis*
*Salix sp*
*Scorzonera humilis*
*Scrophularia nodosa*
*Serratula tinctoria*
*Silene nutans*
*Solidago virgaurea*
*Sorbus aucuparia*
*Succisa pratensis*
*Trifolium medium*
*Trifolium pratense*
*Unspecified*
*Veronica chamaedrys*
*Veronica officinalis*
*Vicia cracca*
*Vicia sepium*
*Viola collina*
*Viola mirabilis*
*Viola sp*

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