



How do changes in environmental conditions affect stress-tolerant and competitive species in wet grasslands.

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Abstract

As a result of changing temperature, water availability and nutrient availability plant diversity is declining. In particular wet grassland species suffer from fluctuating precipitation and competition with other plant species that are better adapted to environmental changes. Recent studies are suggesting that survival of species under such circumstances is dependent on the survival strategy that a species utilizes. In this study I test this hypothesis by using a diverse group of wet, mesotrophic and oligotrophic, grassland species. These species are subjected to a period of 2/3 weeks of drought and shade to simulate the environmental changes. Changes in photochemical efficiency, dryweight/freshweight ratio, rootweight/shootweight ratio, leafweight/stemweight ratio, Specific Leaf Area and stomatal density were measured after the treatment. Plants from different survival strategies were compared under each treatment. This study found that none of the plants adjusted physiological characteristics to the imposed environmental changes. Furthermore no expressed plasticity was found in plants despite differences in survival strategy and treatment. Overall it is concluded that plants barely apply plasticity when they reach an optimal state. This implicates that mesotrophic and oligotrophic grassland communities are more vulnerable for environmental changes rather than competition. The absence of plasticity effects in this study increases the importance of genetic diversity of the plant community. Habitat fragmentation is therefore thought to be a primary cause for the loss of biodiversity. Grasslands are indeed highly fragmented in the Lowlands. Creating sustainable oligotrophic grasslands is thus dependent on restoration efforts. Which in turn are dependent to changes in agricultural land use of the surrounding areas.

Introduction

Climate change effects on ecosystems.

Climate change is expected to have a large impact on plant communities (Thuiller et al., 2005, Walker et al., 2006, Root et al., 2003). Climate changes do so by forcing species to either migrate to other areas of its preferred habitat or to adapt to the newly formed environment. In practice, temperature changes are forcing plant species to relocate to higher latitudes and/or altitudes at a fast pace (Walther et al., 2002, Kelly and Goulden, 2008). Climate change increases productivity and biomass of terrestrial plant ecosystems and individuals (Wu et al., 2011, Xu et al., 2014). This increase, however, cannot compensate for plants unable to disperse and adapt in time (Loarie et al., 2009). Due to the changed environment ecosystems become suitable for new species. Since the previous occupants of the ecosystem were unable to disperse or adapt they now face competition from invasive species better suited for the new ecosystem niche. This competition is expected to result in the displacement and replacement of ecological communities by taxa better adapted to the new environmental conditions (Tilman and Lehman, 2001). Consequently biodiversity decreases in plant communities in areas subject to these changes.

Environmental changes and the effects they encompass for nutrient-poor ecosystems in the Lowlands.

In the past decades the impact of anthropogenic environmental change has grown significantly in size and effect. In particular changes in heavily modified ecosystems such as in the Netherlands and Belgium have experienced large changes in soil composition, climate and disturbance. The Lowlands are situated downstream of the Rhine, Meuse and Scheldt. Its water supply depends largely on precipitation and meltwater from upstream countries (Asselman et al., 2000). Meltwater volume is increasing as a consequence of the temperature increase. Precipitation is expected to increase locally (Klein Tank, 2014) and on a sub-regional level (Jacob et al., 2014) as a result of climate change. Moreover Klein Tank (2014) also predicts prolonged periods of drought during the summer months.

Furthermore the large fluctuations ecosystems in these areas are also subject to eutrophication (Vitousek et al., 1997, Carpenter et al., 1998) which increases plant productivity but is detrimental for biodiversity (Harpole and Tilman, 2007, Silvertown et al., 2006). Nutrient-poor grasslands have been impacted severely through loss of biodiversity and biotope size. Many of these grasslands have been drained and repurposed for intensive agriculture. In the remaining grasslands biodiversity decreased and is thought to have been caused by eutrophication by inorganic fertilisers, re-sowing of plants and extensive cutting or grazing (Bullock et al., 2011). Furthermore it has also been suggested that drainage and lowered ground water levels have influenced biodiversity negatively (Grevilliot et al., 1998). Fluctuation in precipitation and higher water flow from rivers can disrupt groundwater levels and negatively influence biodiversity. Since a stable groundwater level is thought to be beneficial to biodiversity in wet ecosystems (Natuurkennis.nl, 2017).

Mesotrophic and Oligotrophic grasslands.

Throughout the eastern and southern parts of the Lowlands large proportions of mesotrophic sandy soils can be located. In areas in the vicinity of streams and rivers the frequent influx of water has caused these soils to host ecosystems similar to those of wetlands and flood-meadows. Despite anoxia and a fluctuating availability of nutrients, plant biodiversity in these ecosystem is high (Natuurkennis.nl, 2007). In the Netherlands and Belgium a decrease in such mesotrophic and oligotrophic grassland areas has been substantial. In the 19th and first half of the 20th century such lands were used for extensive agriculture in the form of haymaking. Intensive agriculture has recently (latter half of the 20th century) been introduced. This introduction resulted in a decrease in biodiversity of 90% in the UK (Bullock et al., 2011). A similar development can be observed in the lowlands of the Netherlands and Belgium with a decrease in biotope size of blue-grasslands of 99% since 1850 to around 30 hectare (Natuurkennis.nl, 2007).

Plant community changes as a result of climate change.

Ecosystems that experience biodiversity loss often show a shift in dominant plant species (Bakker and Berendse, 1999). The shift to newly dominant plant species is attributed to phenological characteristics. Plant species with characteristics that are beneficial in the newly emerged conditions will increase. Species that lack these characteristics will disappear from the ecosystem (Olf et al., 1997). When traits that are found to have a positive effect on thriving in the new environment are defined, it is possible to predict which species will have an evolutionary advantage. Plants with traits that enable them to cope with the environmental changes occurring in mesotrophic and oligotrophic grasslands are therefore expected to dominate. A further consideration is that the new environment can open up a niche for secondary succession by invasive species. These new species can subsequently introduce additional environmental changes, for example by decreasing nutrient availability or intercepting light (Ehrenfeld, 2010). Competition therefore has the potential to add or enlarge environmental constraints for plants. The biotic and abiotic changes affecting plant communities are shown to cause large disturbances in ecosystems (Bottollier-Curtet et al., 2013), ultimately shifting towards a different ecosystem altogether (Ehrenfeld, 2010).

The influence of environmental changes on the phenology and physiology of plants.

In changing environments plants must adapt or try disperse towards more benign environments in order to survive. However, with the current pace of environmental changes these coping mechanisms can not keep up. Beneficial phenotypic traits become vital for survival when drastic environmental changes happen within the plant's lifespan. Plants can opt to go into a passive state and reserve resources to wait for the environment to become less inhospitable. The short-term character of this strategy, however, makes it for short-lived species such as annuals and biennials often unviable. Since, for these species, the time this strategy requires is needed for growth and procreation. Thus plants are forced to adapt to the new environment by phenotypic plasticity. The process of phenotypic plasticity is described by Valladares et al. (2007) as: "The capacity of a given genotype to render different phenotypes under different environmental conditions." Plasticity can be subdivided into two types: morphological and physiological plasticity. Morphological plasticity describes a plant's ability to form new tissues with new characteristics thereby replacing other kinds of tissue. Physiological plasticity is the spatial rearrangement of subcellular tissue or change in its metabolism which can be reversed by the plant. Morphological plasticity is a high-cost solution while physiological plasticity is less so. However morphological plasticity could provide more benefit to the plant if environmental conditions stay stable. Thus plants face a trade-off for each of the plasticity types. Consequently it can be argued that the plastic response of species affects the longevity and survival in permanently changing environments. Favoring species with higher morphological plasticity over species with higher physiological plasticity. It is, however, important to take into consideration that plants do not possess an optimum amount of plasticity since the high cost of plasticity hinders a high exertion (Valladares et al., 2007). In addition it is meaningful to consider that plasticity is costly under any circumstance. The benefits of plastic responses must therefore outweigh the costs of plasticity. Alpert and Simms (2002) argue that plasticity positively correlates to the predictability of environmental change and that this correlation is codependent on the developmental stage of the plant (Figure 1). Secondly they argue that the duration of the environmental change and the time it takes for the plant to respond is critical in assessing the benefits of plasticity (Figure 2).

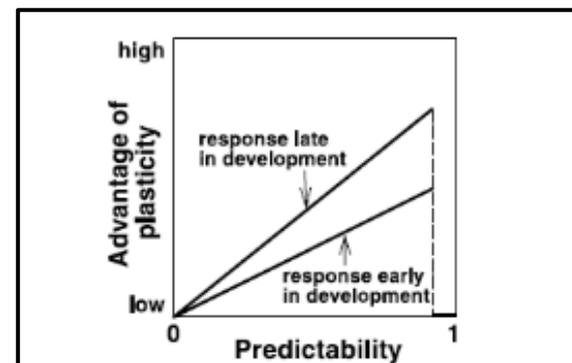


Figure 1. Alpert and Simms (2002): A depiction of the effects of plasticity in relation to response time and the duration of an environmental state

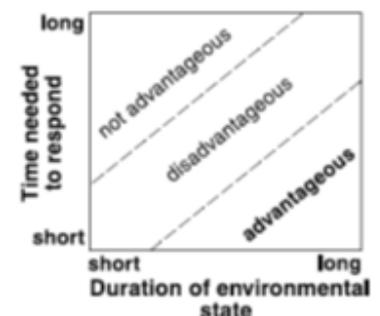


Figure 2. Alpert and Simms (2002): The advantage of plasticity related to the predictability of the environmental changes. Lines refer to the response of environmental developments.

Plant functional traits and their relation to a changing environment.

In practice there is a wide spectrum of physiological, morphological and anatomical adaptations that can be considered phenotypical plasticity responses. Considering changes regarding water and light availability a set of traits can be distinguished that indicates adaptability and competitive ability of plants (Weiher et al., 1999). Shade tolerance is associated with fast growth, allowing plants to outgrow other plants to avoid competition. The capacity to invest relatively more in shoot growth also suggests a high competitive ability for light. Both growth capability and general investment in shoot structures have a high resource dependency. Therefore in environments of low resource availability plants which are slow growing and are efficient in nutrient use have a higher competitive ability than fast growing plants. This correlation between plant functional traits and the environment has been categorized by Grime (1977). Grime distinguishes three plant “strategies” based on a combination of disturbance (“partial or total destruction of plant biomass” (Grime, 1997; p. 1)) and stress (“conditions that restrict production” (Grime 1997, p. 1)) (Table 1).

Intensity of Disturbance	Intensity of Stress	
	Low	High
Low	Competitive Strategy	Stress-tolerant Strategy
High	Ruderal Strategy	No viable Strategy

Table 1. Suggested basis for the evolution of three strategies in vascular plants (Grime, 1977).

Each plant strategy has a set of physiological traits associated to it that optimizes its survival and reproduction. Stress-tolerant species have optimized their physiology for nutrient-efficiency at the cost of slow growth. In oligotrophic and mesotrophic grasslands the stress intensity has decreased due to eutrophication while disturbance levels have stayed low. Meaning that a niche has opened in these grasslands for faster growing Competitive strategists. Due to their fast growth and competitive ability it is expected that Competitive strategists will outgrow Stress-tolerant strategists and outcompete them for resources. Furthermore it is hypothesised that stress-tolerant plants rely more on physiological acclimation whilst competitive plants are able to rapidly change morphological traits (Grime and Mackey, 2002). Plastic response is assumed to be an important mechanism to counteract competitive disadvantages (Grime and Mackey, 2002). The lack of quick plastic responses may therefore accelerate the community shift towards competitive strategists. Under these circumstances it is important to confirm if environmental conditions cause a shift towards a particular strategy and, if so, what conditions cause this shift. How plasticity is expressed in response to these environmental changes and how the effect of the environment can be translated in gains or losses in biodiversity.

This study aims to test the effect of both water and light availability, as they are essential resources subject to change induced by competition (Yang et al., 2011, Valladares et al., 2007). Using three stress-tolerant species, three competitive species and five species that are intermediate between stress-tolerant and competitive strategies. Each species will be exposed to a decreased water availability, a decreased light availability and a control treatment. Addressing three out of four environmental gradients which are considered to cause adaptation as a result of trait variation (Reich et al., 2003). From each of the treatments a set of physiological traits are measured that reflect on the plasticity of each of the species.

Methods

The selection and acquisition of representative grassland species.

To give an accurate representation plants are picked of species that were commonly found in the grasslands and represented each of the plant strategies of interest. A minimum of three representative plant species per strategy(C/CS/S) were picked to decrease the chance that effects of individual species were measured instead of those caused by strategy. For the competitive species *Lycopus Europeaus*, *Phalaris arundinacea* and *Epilobium hirsutum* were cultivated. Five CS-species were cultivated: *Silene flos-cuculi*, *Ranunculus acris*, *Holcus lanatus*, *Pulicaria dysenterica*, *Lotus pedunculatus*. And for the stress-tolerant species *Briza media*, *Carex echinata* and *Mentha pulegium* were cultivated. Seeds of the *L. europeaus*, *S. flos-cuculi*, *R. acris*, *P. dysenterica*, *L. pedunculatus*, *B. media*, and *M. pulegium* were ordered at www.debolderik.net. The seeds from *P. arundinacea* were harvested from wild plants growing in a field on the Zernike campus (53°14'37.4"N 6°31'53.7"E). The seeds from the *E. hirsutum* were harvested from wild plants growing at coordinates 53°14'38.8"N 6°32'08.3"E. Lastly, the seeds from the *H. lanatus* were harvested at the National Park Dwingelderveld in a heath field (52°47'35.9"N 6°23'29.2"E). *C. echinata* plants were grown from shoot cuttings from large individuals that had been cultivated for previous experiments.

Cultivation and treatment of the plants.

Seeds were firstly grown inside a greenhouse in plastic nursery pots (12x12x10cm) filled with substrate from Horticoop (<https://www.horticoop.com/substrate-slingerlandpotgrond/>) and hydrated regularly. Once the seeds sprouted ($\pm 2/3$ weeks) individual plants were re-potted individually into smaller nursery pots (7x7x7cm) filled with Horticoop substrate. The 7x7x7 pots filled with Horticoop substrate were also used for the *C. echinata* cuttings. Of each plant species 40 individuals were cultivated, the smaller pots were placed into a large(40x60x10cm) trays. The trays were filled with water to keep the plants hydrated.

Since the plant species consisted mainly of plants adapted to high groundwater levels or even partial submergence the water level was kept slightly below ground level(the top of the plant containers). The plants were then grown until plants reached a point of full growth, mostly up to a point at which plants started flowering. For most plants except for *B. media* and *C. echinata* this meant being individually re-potted into the larger nursery pots of 12x12x10cm after approximately 3 to 4 months, depending on the species. From the 40 cultivated individuals 10 plants were randomly selected for each of the three treatments and 10 plants were kept as a reserve. The control treatment was set up in the large trays, maintaining high water availability(1 cm below ground level) and exposing plants to the maximum light available in the greenhouse(150-185 $\mu\text{mol}/\text{m}^2/\text{s}$). For the shade treatment plants were placed under a double-layered cheesecloth reducing light exposure to 17.5–22.5 $\mu\text{mol}/\text{m}^2/\text{s}$. Water exposure levels were maintained at the same quantity as for the control treatment. For the drought treatment a test was conducted to determine the maximum duration for which all plants were able to sustain leaves despite lack of water. A period of two weeks of not adding water to the trays was found to suffice. Without adding water to the trays the water left inside the trays evaporated and got absorbed by the soil inside the containers within three days. After 7 days some water was added(a one cm layer/2,4 L) to prevent complete wilting and keep the plants healthy enough to be able to measure. The shade treatment and control treatment were also conducted over a two week period. Besides the maintenance of plant quality for measurements this two week period also allowed the plant to adapt to and grow new tissue in the new environments. This tissue was then used for measuring the adaptability of the plants to the treatments.

The measurement of plant functional traits.

After the treatments the plants were tested for photochemical activity using an IMAGING-PAM Fluorometer (PAM-2000 M-series MINI, Walz, Germany). Before measuring the plants were kept in a dark chamber (5-10 minutes) to ensure that all photosystems were fully dark-adapted. For the measurements the youngest fully-grown leaves were analysed since they developed during the treatment, are likely to have the highest photosynthetic capacity and would therefore be most impacted by the treatments. The top sides of the leaves were then aligned in front of the camera in the 26x38mm frame of the IMAGING-PAM MINI clamp, with the leaves covering at least 50%. The obtained images were then analysed in ImagingWin software. From the obtained images 10 same-size circular areas on the leaves were selected to get an accurate representations of the photosynthetic properties of the entire leaf surface.

To measure the other parameters the plants were washed, getting rid of the substrate around the roots. Plants were split, removing the sprout from the roots for measuring root/shoot ratios. The sprout was split into stems and leaves for the plants in which stem tissue could be distinguished from leaf tissue. If flowers were present on the plants they were measured as being part of the leaves, since the flowers attribute to the photosynthetic production in a more prominent way than stems do (Aschan and Pfanz, 2003). The separated sprout parts were then weighed and used for calculating leaf/stem ratios. From the sprout I then used 10 leaves which had developed during the treatment for measuring specific leaf areas (SLA). After images for the SLA were made with a digital camera the 10 selected leaves were weighed and all of the plant material, sorted for each measurement, was individually stored in paper bags. The samples were then dried in an oven at 80°C for 24 hours and weighed again for calculating fresh weight/dry weight ratios.

Processing and analysis of the obtained images and data.

Since the plant trait measurements required fresh plant material and species to be measured on the same day to maintain similar measuring conditions analysis of the SLA and IMAGING-PAM data was conducted afterwards. For the SLA measurements the images obtained from the camera were used to analyse and calculate surface areas using imaging software ImageJ (Rasband, 1997-2016). The weight measurements mentioned earlier were then used to calculate the SLA.

All of the obtained data was transferred into Microsoft Excel for analysis.

The data obtained from Excel was then exported into R (R Core Team, 2014) for statistical analysis. Since this study was interested in the combined effect of treatment and primary strategy a two-factor ANOVA was performed using a linear model and a Tukey-test to estimate significant differences. This analysis was repeated for each of the measured parameters.

Results

The relation between plant strategies, treatment and mean photochemical efficiency.

After analysis it was found that treatments did not affect mean photochemical efficiency (Table 4). It was found that C-strategy plants have a lower mean photochemical efficiency compared to the other treatments. The intermediate strategy and S-strategy plants did not show significant differences from one another (Figure 3, Table 3). Finally no combined interactions between treatment and strategy were found to have affected mean photochemical efficiency (Table 2).

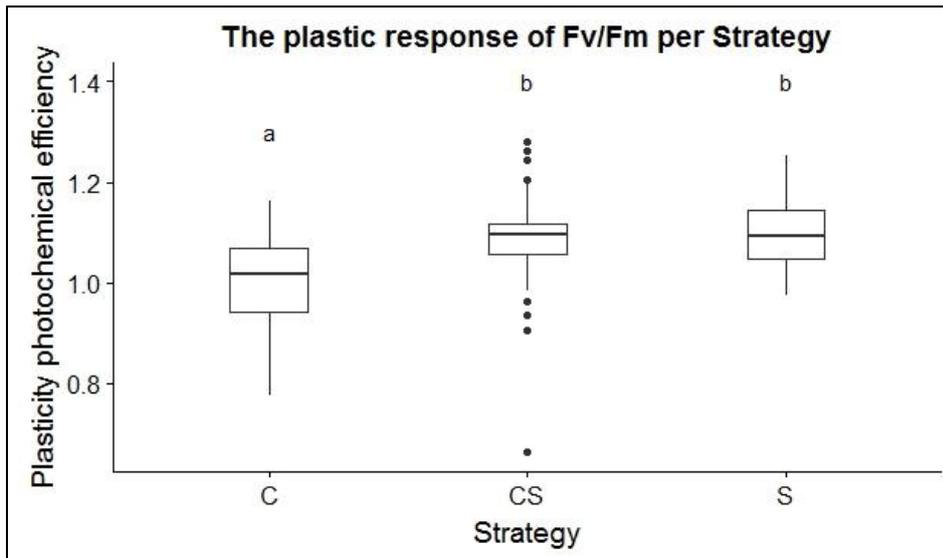


Figure 3. Plastic response in photochemical efficiency(Fv/Fm) of plants from three survival strategies. The values represent proportional changes in mean Fv/Fm using the average response of C-strategists as a benchmark. The C represents competitive strategists(n=90), CS represent intermediate(n=150) and S represents Stress-tolerant strategists(n=89). Letters mark significant differences of $p < 0.05$ from the TukeyHSD-test.

Interactions between plant strategy, treatment and mean Dryweight/Freshweight ratio. Competitive strategies were found to have the highest mean Dryweight/Freshweight ratios. The intermediate and stress-tolerant strategies were found to have significantly lower mean Dryweight/Freshweight ratios similar to each other(Figure 4, Table 3). Treatment was in this case not found to affect mean Dryweight/Freshweight ratios (Table 4). A significant interaction effect of treatment and strategy was also absent(Table 2).

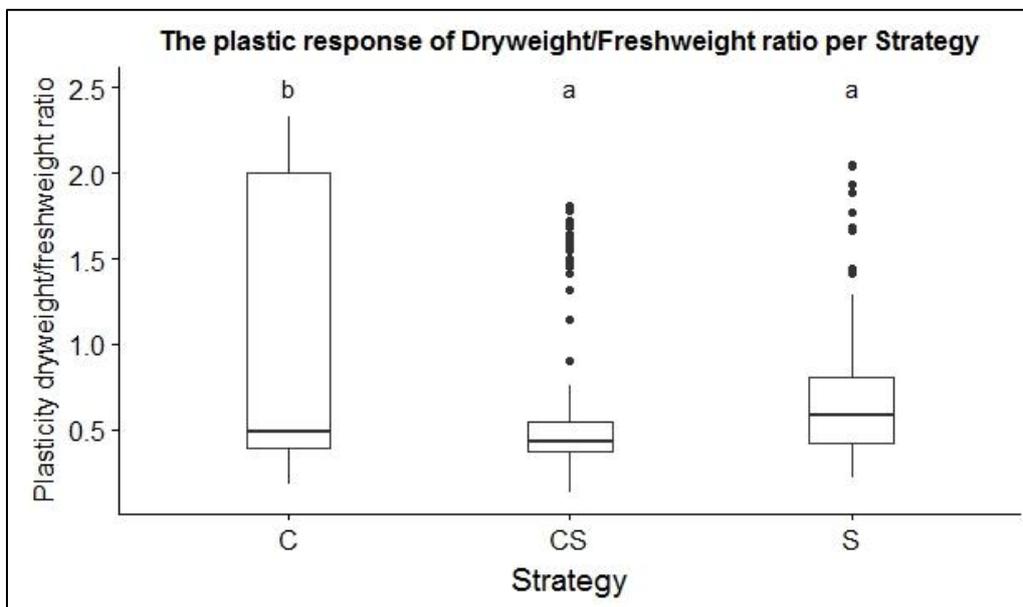


Figure 4. Plastic response in dryweight/freshweight ratio of plants from three survival strategies. The values represent proportional changes in mean dryweight/freshweight ratio using the average response of C-strategists as a benchmark. The C represents competitive strategists(n=90), CS represent intermediate(n=150) and S represents Stress-tolerant strategists(n=89). Letters mark significant differences of $p < 0.05$ from the TukeyHSD-test.

The association between treatment, plant strategy and mean Rootweight/Shootweight ratio.

Plants from intermediate strategies were found to have significantly lower mean Rootweight/Shootweight ratios than the S and C strategists. The S and C strategists were also found to have equal mean Rootweight/Shootweight ratios (Figure 5, Table 3). Mean Rootweight/Shootweight ratios were found to have significant differences for the different treatments (Table 4). The drought treatment showed a higher mean Rootweight/Shootweight ratio than the shade treatment. The control treatment was not found to be different from the other treatments (Figure 6). Interaction effects between treatment, strategy and mean Rootweight/Shootweight ratio were not found (Table 2).

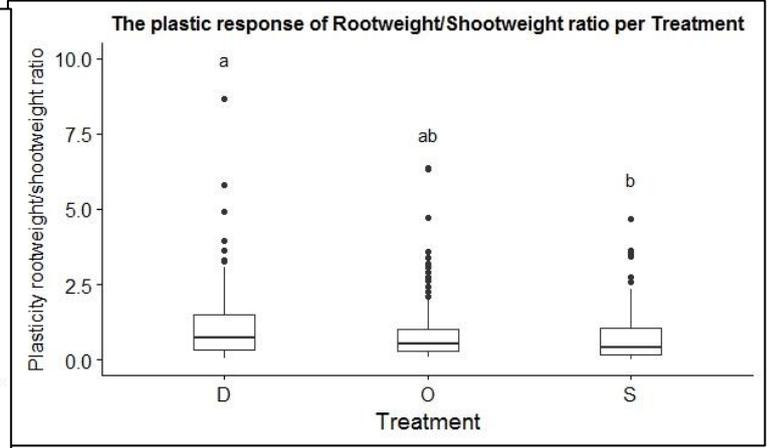
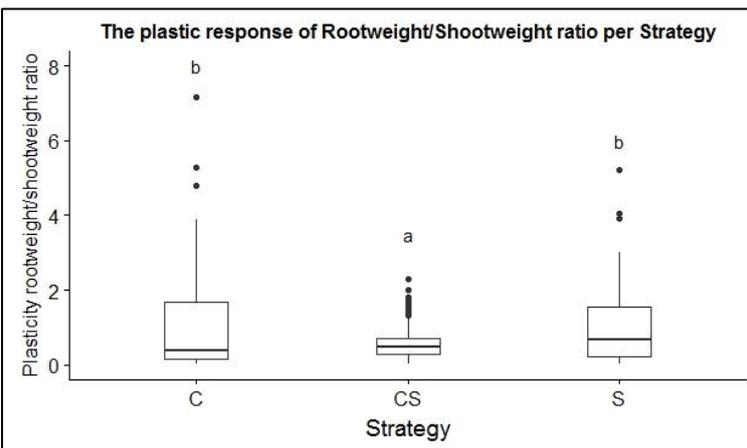


Figure 5. Plastic response in rootweight/shootweight ratio of plants from three survival strategies. The values represent proportional changes in mean rootweight/shootweight ratio using the average response of C-strategists as a benchmark. The C represents competitive strategists (n=90), CS represent intermediate (n=150) and S represents Stress-tolerant strategists (n=89). Letters mark significant differences of $p < 0,05$ from the TukeyHSD-test.

Figure 6. Plastic response in rootweight/shootweight ratio of plants exposed to three treatments. The values represent proportional changes in mean rootweight/shootweight ratio using the average response of the O-treatment as a benchmark. The D represents plants exposed to drought treatment (n=109), O represent plants exposed to the zero-treatment (n=110) and S represents plants exposed to shade treatment (n=110). Letters mark significant differences of $p < 0,05$ from the TukeyHSD-test.

Relationship between strategy, treatment and mean Leafweight/Stemweight ratio.

Plants did not adjust their Leafweight/shootweight ratio significantly to the different treatments (Table 4). The Stress tolerant species had a significantly lower mean Leafweight/Stemweight ratio compared to the intermediate and competitive strategists (Figure 7, Table 3). No significant difference was measured between competitive and intermediate strategists. A combined effect of strategy and treatment on mean Leafweight/Stemweight was not found (Table 2).

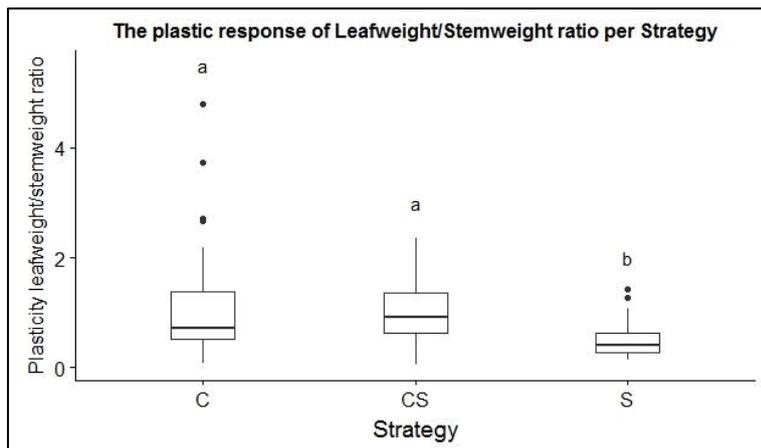


Figure 7. Plastic response in leafweight/stemweight ratio of plants from three survival strategies. The values represent proportional changes in mean leafweight/stemweight ratio using the average response of C-strategists as a benchmark. The C represents competitive strategists (n=90), CS represent intermediate (n=150) and S represents Stress-tolerant strategists (n=89). Letters mark significant differences of $p < 0,05$ from the TukeyHSD-test.

Interaction effects between treatment, strategy and mean Specific Leaf Area.

From the statistical analysis we found no influence of primary strategies on mean SLA (Table 3). It was found that all the plants had increased mean SLA in shade, while drought did not affect mean SLA (Figure 8, Table 4). Interactions between both strategy and treatment on mean SLA were not found (Table 2).

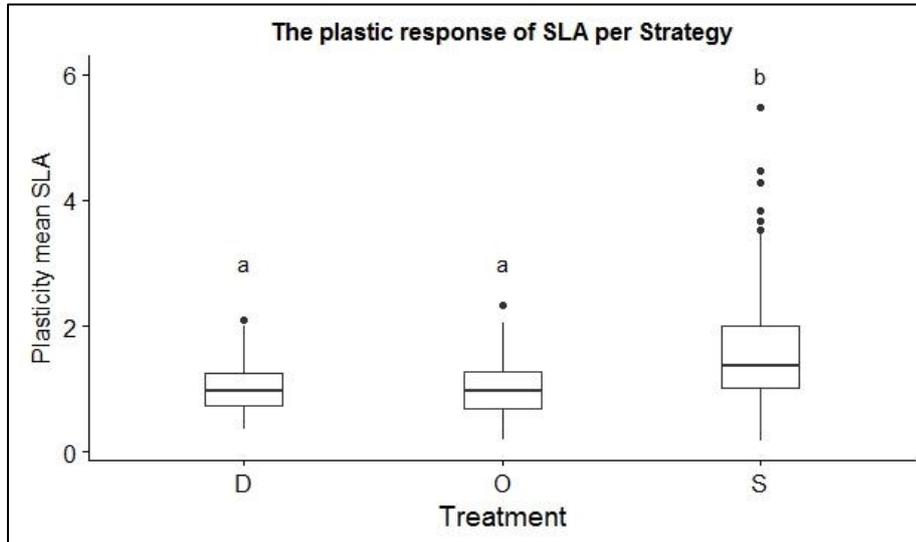


Figure 8. Plastic response in Specific Leaf Area (SLA) of plants exposed to three treatments. The values represent proportional changes in mean SLA using the average response of the O-treatment as a benchmark. The D represents plants exposed to drought treatment (n=109), O represent plants exposed to the zero-treatment (n=110) and S represents plants exposed to shade treatment (n=110). Letters mark significant differences of $p < 0,05$ from the TukeyHSD-test.

The relation between strategy, treatment and mean Stomatal density.

The mean stomatal density on both sides of the leaf was found to not be affected by the treatments (Table 4). The intermediate strategy was found to have significantly lower mean stomatal density on both sides of the leaf (Figure 9, Figure 10, Table 3). Competitive and Stress tolerant did not have significantly different mean stomatal density compared to each other. Stomatal density on both leaf sides was not found to be affected by interaction effects of both treatment and strategy (Table 2).

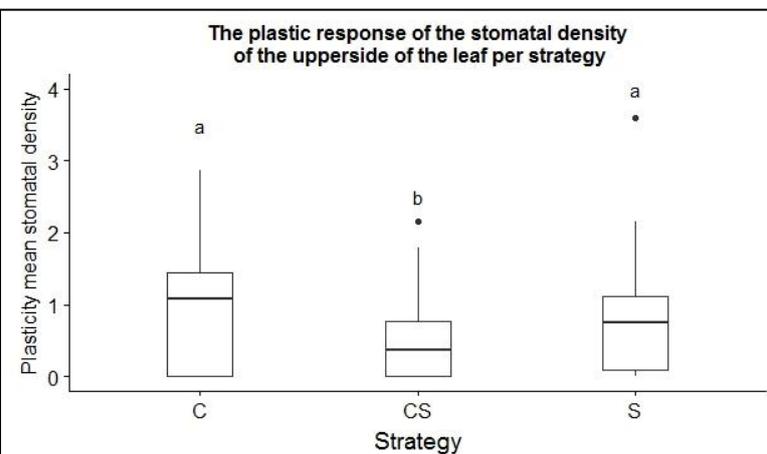


Figure 9. Plastic response in stomatal density on the upperside of leaves from plants from three survival strategies. The values represent proportional changes in mean stomatal density using the average response of C-strategists as a benchmark. The C represents competitive strategists (n=42), CS represent intermediate (n=66) and S represents Stress-tolerant strategists (n=42). Letters mark significant differences of $p < 0,05$ from the TukeyHSD-test.

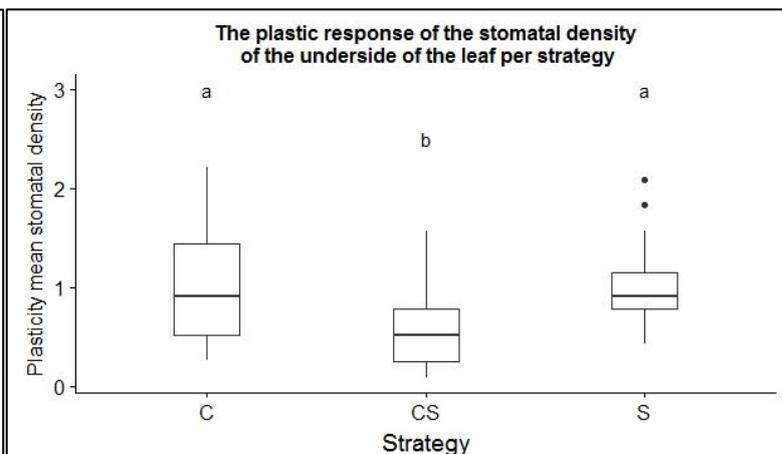


Figure 10. Plastic response in stomatal density on the underside of leaves from plants from three survival strategies. The values represent proportional changes in mean stomatal density using the average response of C-strategists as a benchmark. The C represents competitive strategists (n=41), CS represent intermediate (n=64) and S represents Stress-tolerant strategists (n=41). Letters mark significant differences of $p < 0,05$ from the TukeyHSD-test.

Overview of the results of the statistical analysis

Interaction effects			
Parameter	Df	F-Value	P-Value
Fv/Fm	4	1.6773	0.1550
Dryweight/Freshweight ratio	4	1.6402	0.1639
Rootweight/Shootweight ratio	4	0.2031	0.93657
Leafweight/Stemweight ratio	4	1.0116	0.4025
Specific Leaf Area	4	1.7545	0.1378
Stomatal Density upside of the leaf	4	0.3220	0.8628508
Stomatal Density downside of the leaf	4	0.2527	0.9076

Table 2. An overview of the results of the Two-factor ANOVA on the interaction effects between primary strategy and treatment for each of the measured parameters.

Strategy Effect			
Parameter	Df	F-Value	P-Value
Fv/Fm	2	49.600	< 2e-16 ***
Dryweight/Freshweight ratio	2	15.2208	4.838e-07 ***
Rootweight/Shootweight ratio	2	10.656	3.307e-05 **
Leafweight/Stemweight ratio	2	8.6718	0.0002***
Specific Leaf Area	2	1.6467	0.1943
Stomatal Density upside of the leaf	2	8.6620	0.0002831 ***
Stomatal Density downside of the leaf	2	20.2447	1.982e-08 ***

Table 3. An overview of the results of the Two-factor ANOVA on the effects of primary strategy on the measured parameters.

Treatment Effect			
Treatment Effect	Df	F-Value	P-Value
Fv/Fm	2	2.8289	0.0605.
Dryweight/Freshweight ratio	2	0.7510	0.4727
Rootweight/Shootweight ratio	2	4.4163	0.01282 *
Leafweight/Stemweight ratio	2	2.9177	0.0564.
Specific Leaf Area	2	37.6857	2.01e-15 ***
Stomatal Density upside of the leaf	2	1.5222	0.2218041
Stomatal Density downside of the leaf	2	1.7973	0.1696

Table 4. An overview of the results of the Two-factor ANOVA on the effects of the treatments on the measured parameters.

Discussion

The role of primary strategies and treatment on the expression of plasticity.

In this study it was hypothesised that plants utilising a competitive strategy would be quicker to adapt to a hostile environment than stress-tolerant species. Accordingly it was expected that different plastic responses would be found from each of the strategies when exposed to a treatment. However, the results of the interaction analysis found no such response for any of the parameters (Table 2). The lack of interaction differences can be attributed to either a similar plastic response to treatments by different strategists, or a lack of effect of treatment on plasticity. It is theorised that the high risk and cost that investing in plastic responses brings (Valladares et al., 2007) may explain why the interaction was not found. Exerting plasticity may, for example, leave plants vulnerable to other factors which impose stress on the plant. Plants therefore refrain from using plasticity as a way to counteract environmental stress. Plants may also employ a low-risk type of plasticity capable of adapting to multiple environmental stressors and/or have a low cost. Furthermore plasticity is only thought to be advantageous when the time needed to respond outweighs the duration of the environmental state (Figure 2). The advantage of phenotypic plasticity is further increased when environmental changes become more predictable (Alpert and Simms, 2002) (Figure 1). The predictability of the treatments was low. Because the plants that were measured were grown under optimal conditions and were able to accomplish optimal stage without a necessity to use plasticity. From Figure 1 we can thus conclude that the advantage of plasticity would be very low as well. Another factor that has to be taken into consideration is the ability to accumulate reserve resources in the benign environment (Puijalón et al., 2008). These resources may be used in a later period when the plant has endured the environmental change. *E. hirsutum* for example was observed to mainly invest in surface-level shoots with minimal leaf surface for a minimum of 3 months before investing in photosynthetic shoots. For annual plants this period of build-up may have allowed for enough resources to produce reproductive organs after which there is no need for resources since their lifecycle is about to end. The timing of environmental change related to the developmental stage of the plants therefore plays an important role in the necessity for plasticity. Moreover it is important to include other temporal and ontogenetic effects on plasticity as it has been proven to affect plasticity (Wang et al., 2017). Because the experiment was conducted in a greenhouse any effects that seasonal changes have on the development and corresponding plasticity was largely eliminated. Traits that are specifically adapted to temperature increase/decrease or specific light regimes, that come with seasonal change, were thus not measured. Especially ontogenetic traits such as germination time, growth rate and morphology are tightly matched to seasonal changes. Being able to use plasticity in response to the environmental changes that occur during earlier ontogenetic stages is a great advantage, increasing competitive ability (Fazlioglu et al., 2016).

The effect of plasticity expression on biodiversity and species composition in nutrient-poor grasslands.

Plasticity is a potential driving force of speciation, allowing for a large ecological amplitude through separation of ecotypes and segregation results in new species (Luttge, 2010). A lack of plasticity on the other hand may decrease survivability. A lack of plasticity could be used as a trade-off for other traits that may increase survivability. But when environmental changes become commonplace plasticity may prove essential in surviving (Liu et al., 2017). When considering the methodology of this study the results can best be applied to established communities. Seeing that plasticity was not found to differ between different strategies it is hypothesised that introduced competitive species are not able to outcompete stress-tolerant species when exposed to drought periods or in shade. The possibility that the reoccurrence of environmental events may drive plant plasticity (Alpert and Simms, 2002) must, however, be taken into account. The effects of environmental changes on plant communities and their biodiversity remain unclear since a multitude of factors need further study. As for the information gathered in this study it can be concluded that plants refrain from using, or are unable to use, any amount of plasticity. This becomes apparent when the results are compared

to other studies on plastic responses (van Kleunen et al., 2007, Lambrecht et al., 2017). This study has proven, however, that differences in plasticity between primary strategies are minor and are not indicative for biodiversity patterns and extinction rates.

A revision of environmental change and its effect on the sustainability of nutrient-poor ecosystems in areas of intensive land use.

This paper focusses on plasticity as a means to negate and overcome environmental hazards. As plasticity depends on a large genetic diversity the topic of gene flow in these ecosystems is frequently touched upon. Selection pressure is steadily increasing with the current velocity of environmental changes (Loarie et al., 2009). Having a wide genepool in these situations increases chances for a species to find an adaptive solution to survive. On the contrary small genepools promote inbreeding and compromise the ability of ecosystems to adapt (Leimu et al., 2010). Habitat fragmentation is therefore considered to be particularly destructive to the adaptive ability of ecosystems (Jump and Penuelas, 2005, Matesanz and Valladares, 2014). As is touched upon earlier in the introduction habitat size of nutrient-poor ecosystems has decreased dramatically in the Lowlands and is accompanied by fragmentation as well. Considering the minor expression of plasticity that was found in this study it is hypothesised that habitat fragmentation plays a more important role in the loss of biodiversity (Habel et al., 2013). When the issue of sustainability is addressed in the Lowlands a focus on habitat expansion and connection may enlarge the genetic pool. Currently restoration strategies for these areas focus on restoring high water levels and introducing litter from other grasslands to introduce genetic diversity (Beije et al., 2012). Eutrophication in these areas remains unavoidable so increased mowing and removing top soil slabs are the only possibility to siphon off nutrients. Adjacent grasslands can greatly improve the success of restoring former grasslands. Current projects show that restoration times range from 10 to 20 years. However, restoration success heavily depends on the influence of nearby agricultural land use.

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