Ecological differentiation of *Carex* species coexisting in a wet meadow: comparison of pot and field experiments

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Abstract

Competitive exclusion is to be expected between phylogenetically similar species that share traits and resources. However, species may overcome this, either through differentiation of their responses to biotic and abiotic conditions, or by trait differentiation, thus enabling their coexistence. We identified differences in phenotypic traits between seven coexisting *Carex* species and their responses to competition and fertilization in pot experiments, before using long-term field experiments to generate responses of the *Carex* species to fertilization and mowing and to illustrate temporal variability between species. Finally, we assessed how effective the results of the pot experiment were at predicting species responses in the field. In pot experiments, we found that species responded more to competition than to fertilization. Notably, all species showed similar responses to these factors in the pot experiments. Fertilization decreased the root:shoot ratio, whilst competition decreased growth-related characteristics such as total biomass, irrespective of the species. Differences among species were only found in their clonal response to competition, namely rhizome production and generation rate of new ramets. These findings support the idea that different clonal growth strategies may facilitate niche partitioning of *Carex* species. Species responses measured from pot experiments were poor predictors of their responses in the field experiment. Nevertheless, we confirmed the prediction that, over time, *Carex* species with lower growth rates in pot experiments showed more stable biomass production than in the field. We suggest that differences in clonal traits and temporal dynamics support the ability of *Carex* species to avoid competitive exclusion, enabling their coexistence.

Keywords: *Carex*, coexistence, clonality, competitive exclusion, nutrients, limiting similarity.

1. Introduction

Several mechanisms have been proposed to explain how similar species coexist, yet this question remains central to ecology (Palmer 1994, Vellend 2016). Following Hutchinson (1959), MacArthur and Levins (1967) introduced the concept of limiting similarity. This predicts that, for similar species to coexist, there should be some minimum difference in species resource utilization. For example, coexisting species should not completely overlap in the niches they occupy or in their responses to biotic and abiotic conditions. Plants use a very limited number of resources, and yet, at small spatial scales, species richness can be high, with several similar species competing for the same resources (Wilson et al. 2012, Chytrý et al. 2015). Whereas we can expect a high degree of overlap among resource utilization of similar species, we may also expect some differences in resource use, and particularly resource utilization curves (see below) that would enable species coexistence (Palmer 1994).

Phylogenetic conservatism would also predict that closely related taxa, such as congeneric species, share similar life-history traits and therefore resource-use capacities (Prinzing et al. 2001, Davies et al. 2013). Indeed, Charles Darwin (1859, p. 111) stated that closely related species are similar in their morphology and ecological requirements, increasing the intensity of competition among them. In an apparent breakdown of the predictions of competitive exclusion, how are closely related species, sharing similar resources, able to coexist?

The genus *Carex* consists of a high number of species that occupy a diversity of habitats, ranging from open grassland to forests (Waterway et al. 2009). In a species-rich wet meadow in Ohrazení (Lepš 2014) where long-term experimental plots are located, several *Carex* species are regularly found coexisting at very fine spatial scales. Niche segregation is not uncommon among closely related coexisting wetland species. However, this usually occurs along environmental gradients such as soil moisture or acidity (Waterway et al. 2009). During intensive sampling campaigns of field plots in Ohrazení, up to four *Carex* species were found to coexist in a single 10 ×10 cm grid cell (i.e. small enough to be environmentally homogenous); 2–8 *Carex* species were regularly found in 1 m^2 plots (which, in the mown unfertilized variant, contained up to 40 vascular plant species), while a total of 10 Carex species were found in the 1 ha site among more than 100 vascular plant species (Lepš 2014). Similarly, in Laelatu wooded meadow (Estonia, one of the most species rich meadows

in the world, Wilson et al. 2012), five *Carex* species were found in a 20×20 cm grid cell (Kull & Zobel 1991). Previous studies from the Ohrazení site demonstrated that the number of species, including *Carex*, decreased with fertilization (Lepš 1999). Application of fertilizer almost immediately increased total biomass of the community, which in turn increased competition for light, suppressing weaker competitors (Lepš 2014). Although this held true for most *Carex* species, other members of this genus displayed a range of responses to fertilization (Lepš 2014), suggesting that even small differences in responses to competition and fertilization can contribute to coexistence among *Carex*.

Resource utilization curves describing variation in the growth of species along resource gradients are used to define limiting similarity (MacArthur & Levins 1967). When unknown, as is more often the case, resource utilization curves have to be determined experimentally. A greater range of variation in the response of closely related species along such gradients implies lower similarity of resource use, offering a potential explanation for their coexistence. For example, where there is low heterogeneity of biotic and abiotic conditions within a meadow community, differences in the traits associated to timing and type of clonal growth could enable species coexistence. *Carex* species display a variety of clonal traits and strategies (Krahulec 1994). Some species can respond to environmental heterogeneity by varying the amount of rhizome branching and rhizome length (de Kroon & Hutchings 1995). This so called “foraging behavior” enables these species to avoid unfavorable patches of soil while positioning their rhizomes or stolons in areas of high nutrient availability. For example, *Carex flacca* may overcome fine-scale variation in resource availability by transferring resources between connected rhizomes (de Kroon et al. 1998).

Another potential coexistence mechanism is the storage effect which states that no species can thrive under all conditions and that different species use a range of coping strategies under changing conditions to ameliorate against bad years (Cáceres 1997, Angert et al. 2009). For example, the temporal coefficient of variation (CV) in biomass of a species with a high relative growth rate (RGR) is likely to fluctuate more across favorable and unfavorable years, profiting from ‘good’ years and declining more in ‘bad’ years. Alternatively, populations of species with lower RGR tend to be less sensitive to annual fluctuations in conditions (have lower temporal CV), showing buffered population growth (Chesson et al. 1981, Kelly et al. 2002). It is therefore possible that the coexistence of congeneric species, such as *Carex*, could be

supported by differing RGRs, each predicting different patterns of temporal stability (Májeková et al. 2014).

In an attempt to improve our understanding of coexistence of closely related species we used the example of *Carex* to ask the following questions: (i) Do phenotypic responses of coexisting *Carex* species to nutrient availability and competition vary among species in a short-term pot experiment? Since the findings of pot experiments may not provide a realistic prediction of how *Carex* species respond in field experiments, we also ask (ii) whether the differences in species traits and responses found in pot experiments predict the long-term performance and temporal fluctuations of *Carex* species in a long-term field experiment. We compared the results of the pot experiments to the first four years of data from our long-term experiment which provided values for the initial responses to treatments (Lepš 1999). The long-term responses to treatments were characterized by species abundances 20 years after the start of the experiment (Lepš 2014) and also by species temporal variability (Májeková et al. 2014).

2. Materials and methods

2.1. Study material

The genus *Carex* (Cyperaceae) is a species rich genus in Central Europe (and in temperate flora in general). The following *Carex* species were used in this study: *C. demissa* Hornem, *C. hartmanii* A. Cajander, *C. pallescens* L., *C. panicea* L., *C. pilulifera* L., *C. pulicaris* L. *and C. umbrosa* Host. Other *Carex* species present in the locality are: *C. leporina* L., *C. echinata* Murray and *C. nigra* (L.) Reichard. These species were not included in this study because we were not able to collect a sufficient number of their ramets. *C. leporina* and *C. echinata* are rare at the locality and *C. nigra* is very difficult to identify in early April, when the ramets were collected (see below). *Carex* rhizomes typically branch sympodially. Among our focal species, *C. hartmanii* and *C. panicea* are able to produce numerous long horizontally creeping rhizomes (particularly in *C. hartmanii* where the length of the rhizome branch can reach about 0.5 m – Appendix 1). *C. demissa*, *C. pallescens* and *C. umbrosa* possess only very short rhizome branches. *C. pilulifera* and *C. pulicaris* form frequent but rather short

rhizome branches. To characterize the type and extent of rhizomes of our focal *Carex* species, we excavated one rhizome system per species from our experimental site in Ohrazení, mapping the position of individual ramets and length of spacers (see the schematic in Appendix 1 for further detail).

On April 2, 2001 and April 4, 2002, ramets of each species were taken from the Ohrazení site for use in the pot experiments, where growth responses to nutrient availability and competition respectively were tested. Each ramet consisted of a young vegetative rosette with several young roots. Initial individual size was recorded in order to calculate their responses.

2.2. Fertilization pot experiment

In the fertilization experiment, plants were grown in pots (upper diameter 16 cm, lower diameter 10 cm, height 15 cm, volume 2 liters) containing substrates with low, medium and high mineral nutrient levels. The basic (low nutrient) substrate consisted of a mixture of commercially sold peat and sand (in 1:2 ratio). The medium and high nutrient substrates were created by adding 1 g and 4 g of commercial NPK fertilizer (19 % N, 6 % P, 12 % K) respectively, to the basic substrate in each pot. There were five pots of each substrate type, containing one ramet per pot of each species, totaling 105 pots. Potted plants were grown for 96 days in a greenhouse at the University of South Bohemia, Czechia. The final design was not fully balanced due to some mortality and preliminary misidentifications of ramets in early spring that were later corrected when individuals were more developed.

Before planting, the fresh weight of ramets was recorded. At the end of the experiment, all plants were harvested, and we counted the number of individual ramets and rhizomes, recorded the fresh and dry biomass (accuracy 0.01 g) after drying for 24 h at 80 °C, separated above ground parts into leaves and flowering stems and belowground parts into roots and rhizomes. Based on the regression of the dry weight on the fresh weight recorded at the end of the experiment, we estimated the dry weight of each ramet at the beginning (separate regression for each species). Then we used this value to calculate the relative growth rate (RGR) as

$$\text{RGR} = \frac{\ln(DW_t) - \ln(DW_0)}{t}$$

where DW_t is the dry weight of the whole ramet at the end of the experiment, i.e. in time t (i.e. time of duration of the experiment, i.e. 96 days), and DW_0 is the dry weight at the start of the experiment. The values of RGR are thus in [days^{-1}]. Individuals that were established but died (in the competition experiment), or decreased their weight, were assigned an RGR of 0. From the above- and belowground parts of the final biomass we calculated the root:shoot ratio as the dry weight biomass of roots (i.e. belowground resources acquisition structure, thus excluding the rhizomes) divided by the dry weight of leaves (i.e. photosynthetic structure). Dry biomass weight of a dead individual were recorded as zero, and the height and root:shoot ratio were considered as missing values.

2.3. Competition pot experiment

For the competition pot experiment, another set of ramets was prepared as above, and planted into low nutrient substrate. The treatments of no-, moderate, or high intensity of interspecific competition were achieved by using a single *Carex* ramet for no-competition, by sowing 15 seeds of *Holcus lanatus* L. with the ramet for moderate competition, and 45 seeds of *H. lanatus* to achieve high competition. Following germination, the seedlings of *H. lanatus* were thinned to 5 and 15 for the moderate and high competition treatments respectively. Again, for each combination of species and competition level, five replicates were used, totaling 105 pots. Some individuals subsequently died (various species, two in low, three in medium, and two in high competition), leading to a slightly unbalanced design. Plants were allowed to grow for 98 days. *H. lanatus* was selected as a competitor, because the species is common at the site and thus is an important competitor in the field. Based on experience from our previous experiments, *H. lanatus* germinates easily and grows quickly (including clonal spread), which is important in a relatively short-term experiment.

Following the previous experiment, fresh biomass of each ramet was weighed before the experiment. During the experiment, the number of ramets was counted at 28, 52, 78 and 98 days. At the end of the experiment, plants were harvested allowing

measurement of fresh and dry, as well as above- and belowground biomass. RGR and root:shoot ratio were calculated as detailed above and the numbers of belowground rhizomes were counted.

2.4. Field experiment

As discussed in the introduction we used data from a field experiment of Lepš (1999, 2014) and Májeková et al. (2014). The study site is located near Ohrazení, 10 km South-East of České Budějovice (48° 57´ N, 14° 36´ E, 510 m a.s.l.), with 7.8° C mean annual temperature and 620 mm mean annual rainfall (local meteorological station). The site is a species-rich oligotrophic wet meadow, traditionally managed by mowing, once or twice a year. Species composition corresponds to Molinion caeruleae, with some transitions to Violion caninae. None of the *Carex* species can be considered dominant species in the study area, but *C. hartmanii* and *C. panicea* achieve cover above 15% in some parts of the meadow.

In 1994 a long-term experiment was established at this study site, combining the fertilization, mowing and removal of dominant, *Molinia caerulea* (L.) Moench. The experiment was set in a factorial design with each of the eight possible combinations replicated in three, 2 × 2 m plots (24 plots altogether). The results and detailed description of the design of this experiment and monitoring regimes have been previously published as follows. The results (development of species composition) against which we compare species performance in the pot experiments are published in (1) Lepš (1999) which describes the immediate response of community composition to the introduction of the treatments. These data were also analyzed and used as a training set in the chapter 15 of Šmilauer and Lepš (2014) textbook on multivariate data analysis. (2) Lepš (2014) provided a summary of vegetation development during the first 15 years. (3) Májeková et al. (2014) detailed the temporal variability of biomass of individual species.

In this paper, we use three characteristics of individual *Carex* species derived from the field experiment: (1) the immediate response to mowing and fertilization at the beginning of the experiment, (2) the long-term responses based on species composition in 2014 (i.e. 20 years after the start of the experiment), and (3) the variability of biomass of individual species over a 13 year period, expressed as a

coefficient of variation (CV). We only used the responses to fertilization and mowing, discarding the removal of the dominant treatment, because only species composition showed a significant responce to them.

2.5. Data analysis

2.5.1. Pot experiments

We analyzed the response of individual species to different levels of fertilization and competition with a two-way analysis of variance (ANOVA), considering the following response variables: relative growth rate (RGR), root:shoot ratio, height, number of ramets, total dry weight, and number of rhizomes. Most variables were measured at the end of the experiment, with the exception of total dry weight of *C. pilulifera* with No fertilization treatment (only one replicate due to mortality) for which the average value of the various individuals of *C. pilulifera* from no competition treatment (virtually identical) was assigned. The values for total dry weight were log-transformed (to cope with the zero values for individuals that died, we have used log(x+1)). In the fertilization experiment, we tested the main effect of species identity, level of fertilization and their interaction and, in the competition experiment, the effect of species identity, level of competition, and their interaction. When testing the effect of the treatments on number of rhizomes, the species that did not produce any rhizomes were excluded from the ANOVA (although they are maintained in figures for the purpose of visualization).

To measure the number of ramets in the competition experiment, we conducted 4 counts over the experiment. This data was analyzed with a repeated measurement ANOVA, using the number of ramets as a response variable. Species identity, level of competition, time, and their first- and second-order interactions, were used as explanatory variables. In all the ANOVAs, the significant interaction species by treatment signifies that the response to the treatment differs among species. In repeated measurement ANOVA, the interaction species by time means different timing of increase of the number of ramets among species, and the second order interaction (species × treatment × time) shows that species differ in the temporal dynamics of their response to the treatment.

Because the individual response variables were not independent, and because we carried out a separate ANOVA for each of them, there is a danger that some of the significant results might be just due to Type I error. Consequently, we decided to further provide a multivariate common test for all characteristics. We used Redundancy Analysis (RDA; Šmilauer and Lepš 2014), with the five characteristics available for all species as response variables (i.e. relative growth rate (RGR), root:shoot ratio, height, number of ramets, log of total dry weight), species and treatment (i.e. either nutrients, or competition level), as predictors. All the response variables were centered and standardized. Analyses corresponding to the main effects in ANOVA are partial RDAs, with one factor being the explanatory variable, and the other, the covariable. The analysis testing the interaction is obtained by partial RDA, with the interaction being the explanatory variable, and both the main effect being the covariables. The ordination diagrams also indicate correlation between individual response variables.

2.5.2. Field responses

We calculated the field responses of *Carex* species by means of multivariate analyses (RDA), using species composition, characterized by cover of individual species, estimated in the central 1 m^2 quadrats in the 2 × 2 m plots under different treatments. For the estimate of immediate response to the treatment, the data from the first four years of the experiment were used. The first year provided the baseline data, i.e. before any introduction of the treatments, thus, the interaction with time (as a quantitative variable, 0 for the baseline, and 1, 2 and 3 for subsequent years) is the best explanatory variable during the early years. Thus, the interaction: Time × Treatment Under Consideration, was the only explanatory variable, while: Plot Identity, Time, and Time × Other Treatments were the covariables in the RDA on the covariance matrix. The scores of individual species on the constrained axis equate to the characteristics of the individual species' response to the treatment under consideration. This method follows an example of multivariate counterpart of repeated measures ANOVA detailed in Šmilauer and Lepš (2014, chap. 15).

To characterize the response 20 years after the start of the experiment (when the difference among treatments had stabilized), we used the 1 m^2 cover data from 2014. The treatment under consideration was the only explanatory variable used, while the other two treatments were designated as covariables in the RDA. Scores of

individual species on the single constrained axis represented how the species responded to the treatment in question. In both cases, positive values indicated a positive response to the treatment (i.e. either the species increased at different rates during the first four years, or the species was more abundant after 20 years of exposure to that treatment.

Finally, in the mown plots, we evaluated how biomass varied between species and with time (biomass is not applicable from unmown plots). Biomass was measured over the 13 years and its variability was characterized by the coefficient of variation (CV = standard deviation / mean). Both standard deviation and mean were calculated for each 0.25 m^2 plot over 13 years and averaged across the whole site. Species that appeared infrequently were excluded to avoid overestimating CV, as increased measurement error would skew apparent variability. The included species were present in at least six (out of twelve) plots and had an average biomass > 0.002 g per plot. Species with an average biomass < 0.002 g were included if they were found in at least nine plots. During the first seven years, variation of biomass of individual species was governed by directional changes (Lepš et al. 2019) and so these years were omitted, thus, CV should only reflect non-directional variability (see Májeková et al. 2014 for further details).

2.5.3. Predicting field response

Finally, we attempted to predict the short and long-term responses of species to fertilization and mowing. We also attempted to predict the temporal variability of individual species using the species responses determined by pot experiments. Setting the scores of individual species along the constrained axes as response variables, we used the species response variables from the pot experiments as the explanatory variables: relative growth rate, root:shoot ratio, height, number of ramets, total dry weight and number of rhizomes. We considered, as explanatory variables, both the mean values of the traits per species, and the difference in trait values between treatment levels. The means were calculated as the averages of the trait values in control pots of both the experiments (i.e. no fertilization and no competition). Differences were calculated separately for fertilization and competition pot experiments, by subtracting the mean trait values in the respective control from the

trait values in the highest treatment level (i.e. high nutrients or high competition). Therefore, we had three different explanatory variables: differences in fertilization pot experiment, differences in competition pot experiment, and trait averages. In order to explore the data, we selected the best explanatory variable (i.e. the lowest AIC) for the five characteristics mentioned above (i.e. short- and long-term response to fertilization and mowing, and variability in time), and then tested significance using linear regression.

The number of *Carex* species in our locality was limited and not all species were abundant enough to reliably estimate their responses. For this reason, we could not provide robust field data for all the species used in the pot experiments. Given the limited sample of species (n = 7), caution should be taken when considering the power of the statistical tests, which is clearly very low. Moreover, it should be noted that there were many different predictors obtained from the pot experiment, and that the best predictor was always selected for each of the responses. Therefore, caution should also be taken when considering the p-values and thus the ecological significance of our conclusions.

The univariate analyses were carried out in R (R Core Team 2019), and the RDA in Canoco5 (ter Braak and Smilauer 2012). Univariate models were validated on the basis of the distribution of residuals (Appendix 2).

3. Results

3.1. Pot experiments

Species differed in all measured variables both in the fertilization and competition pot experiments (species effects, Table 1, Figure 1 and 2). However, these differences were not markedly modified by fertilization and competition. Almost no significant interactions between species and treatments (in their response to fertilization and competition) were detected ($p > 0.05$), indicating a similar species response to these factors (Table 1). Nutrient availability had the effect of significantly decreasing the root:shoot ratio and had a close to significant positive effect on RGR. Both these patterns were similar across all species. There were no significant species-treatment interactions. Competition significantly decreased RGR, number of ramets, and total dry

weight. The only significant interaction between species and competition was apparent for the number of rhizomes (Table 1B, Figure 2), i.e. the variable used for the rhizomatous species, and not included in the RDA analyses.

[Table 1 here – as it is a large table, it is at the end of the main text, after references]

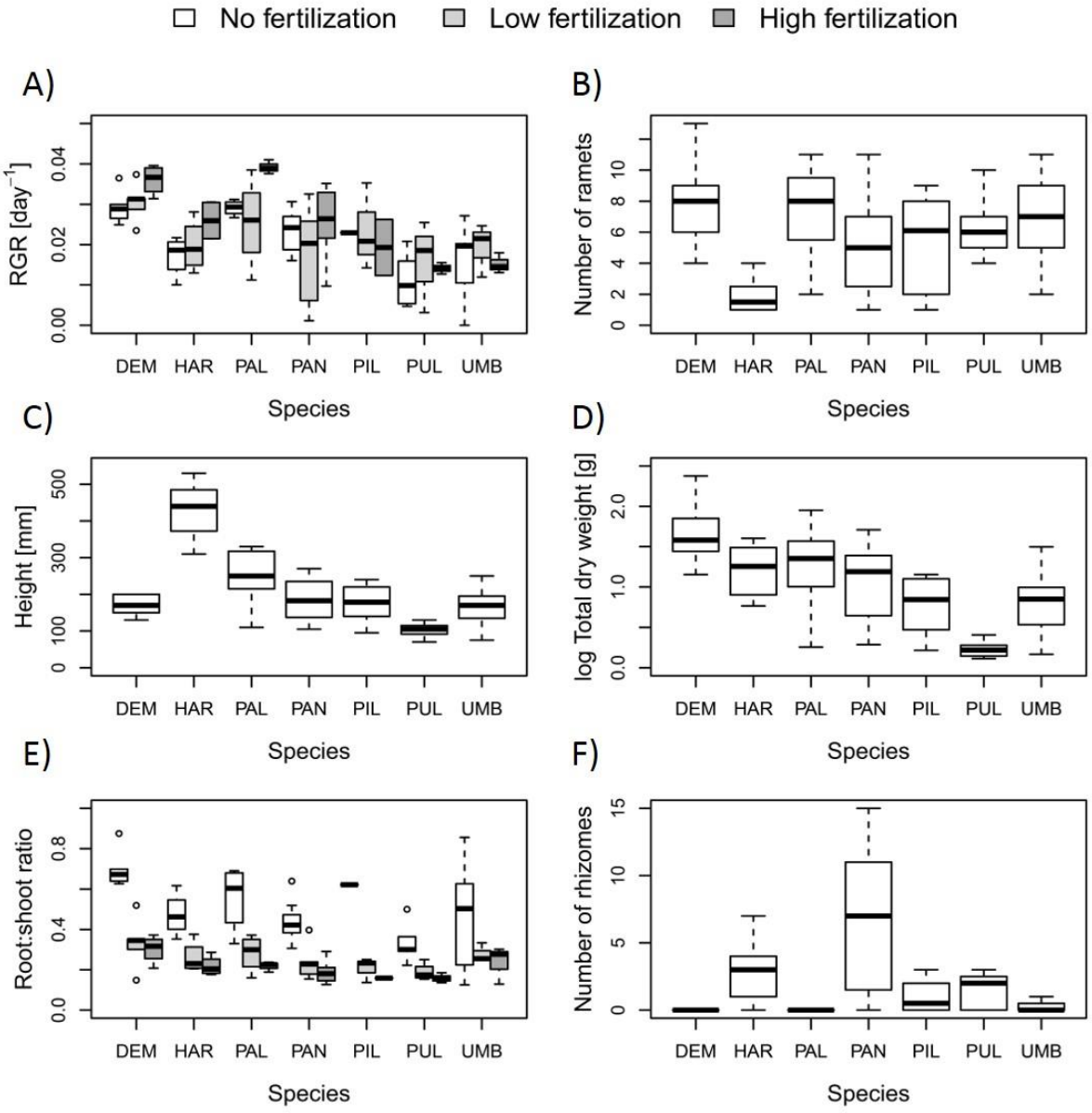

Figure 1. Response of the seven selected *Carex* species to different levels of fertilization. Graphs with a single boxplot are shown where there are no significant differences between treatments, and the average values of all treatments are presented. Multiple boxplots indicate significant (or close to significant) differences between treatments. The measured variables: (A) Relative growth rate, (B) Number of ramets, (C) Height, (D) Total dry weight in logarithmic scale, (E) Root:shoot ratio and (F) Number of rhizomes. Results of Two-Way ANOVA are shown in Table 1. (DEM – *C. demissa*, HAR –

C. hartmanii, PAL – *C. pallescens*, PAN – *C. panicea*, PIL – *C. pilulifera*, PUL – *C. pulicaris*, UMB – *C. umbrosa*)

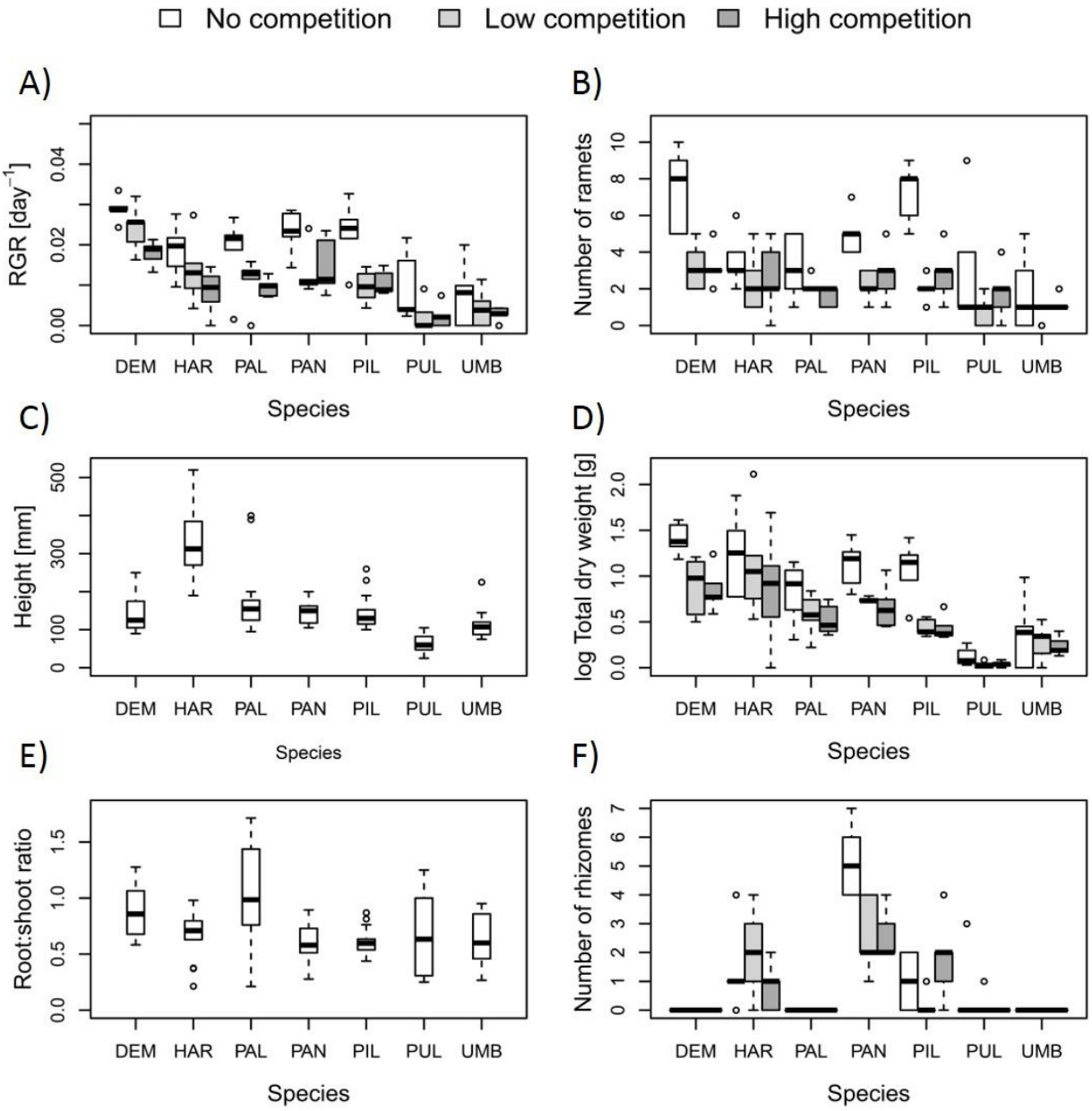

Figure 2. Response of the seven *Carex* species to different levels of competition. Graphs with a single boxplot are shown where there are no significant differences between treatments, and the average values of all treatments are presented. Multiple boxplots represent significant differences between treatments. The measured variables were (A) Relative growth rate, (B) Number of ramets, (C) Height, (D) Total dry weight in logarithmic scale, (E) Root:shoot ratio and (F) Number of rhizomes. (DEM – *C. demissa*, HAR – *C. hartmanii*, PAL – *C. pallescens*, PAN – *C. panicea*, PIL – *C. pilulifera*, PUL – *C. pulicaris*, UMB – *C. umbrosa*)

In the repeated measures ANOVA, the number of ramets differed between species, changed with time, and was affected by the level of competition ($p < 0.01$ for

all). There were also significant first and second order interactions (Table 2, Figure 3). The response of *Carex hartmanii* to competition was an increase in number of ramets toward the end of experiment. In most other species, competition had the opposite effect.

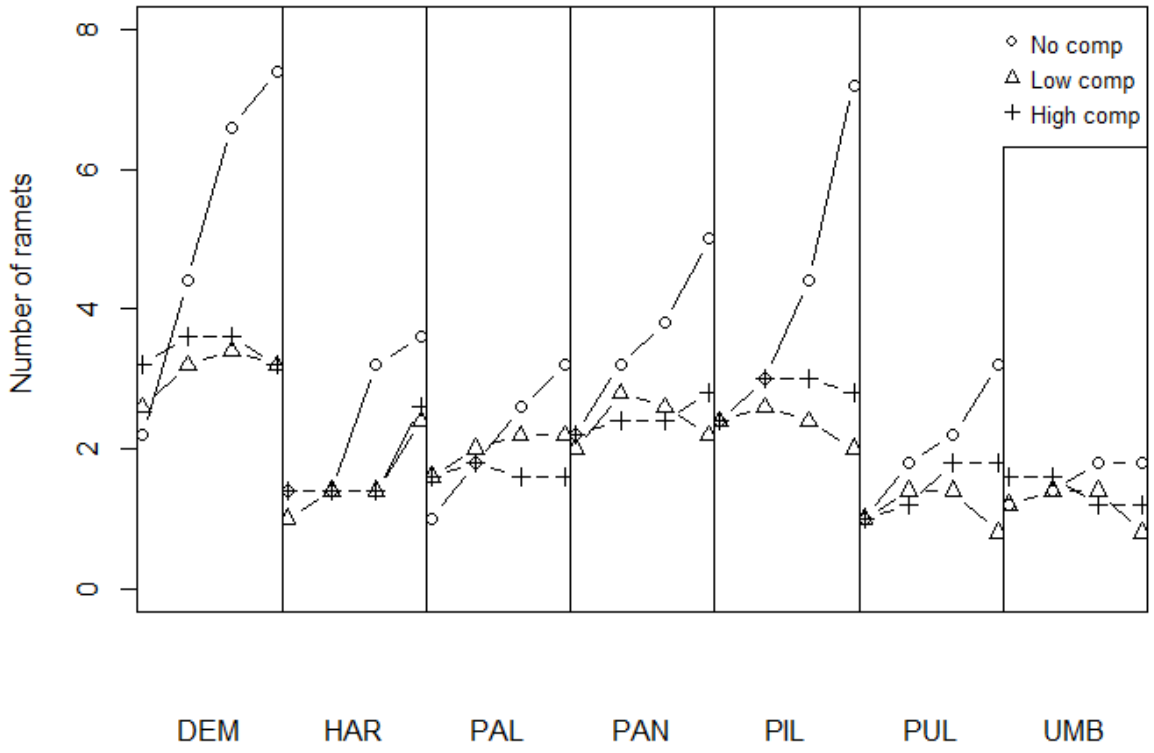

Figure 3. Ramet production over the 98 day time period in *Carex* species in control pots at high and low levels of competition. (DEM – *C. demissa*, HAR – *C. hartmanii*, PAL – *C. pallescens*, PAN – *C. panicea*, PIL – *C. pilulifera*, PUL – *C. pulicaris*, UMB – *C. umbrosa*)

Table 2. Results of repeated-measurement ANOVA for number of ramets of *Carex* species. The bold numbers indicate significant effects ($p < 0.05$).

	Error: Between (df = 84)		
	df	*F*	*p*
Species	6	14.796	**< 0.001**
Competition	2	12.948	**< 0.001**
Species:Competition	12	0.867	0.583
	Error: Within (df = 252)		
	df	*F*	*p*
Time	3	41.879	**< 0.001**

Species:Time	18	2.614	**< 0.001**
Competition:Time	6	24.535	**< 0.001**
Species:Competition:Time	36	2.003	**0.001**

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450 In all cases, the multivariate analyses (RDA) agreed with the result of the
451 univariate analysis of the respective characteristic. In both experiments, there were
452 significant differences among species, but also significant differences between
453 treatment levels (i.e. both, nutrients and competition), in all of them with $p < 0.001$.
454 Therefore, it is unlikely that the significant effects of these two main factors in the
455 univariate analyses (i.e. ANOVA) were a consequence of Type I errors. The amount of
456 variability explained by competition was considerably higher than that of nutrients
457 (Appendix 3). Notably, the species × treatment interaction was not significant in either
458 experiment. The results also show that most variables were positively correlated, with
459 exception of root:shoot ratio. The detailed results of the multivariate analyses are in
460 Appendix 3.

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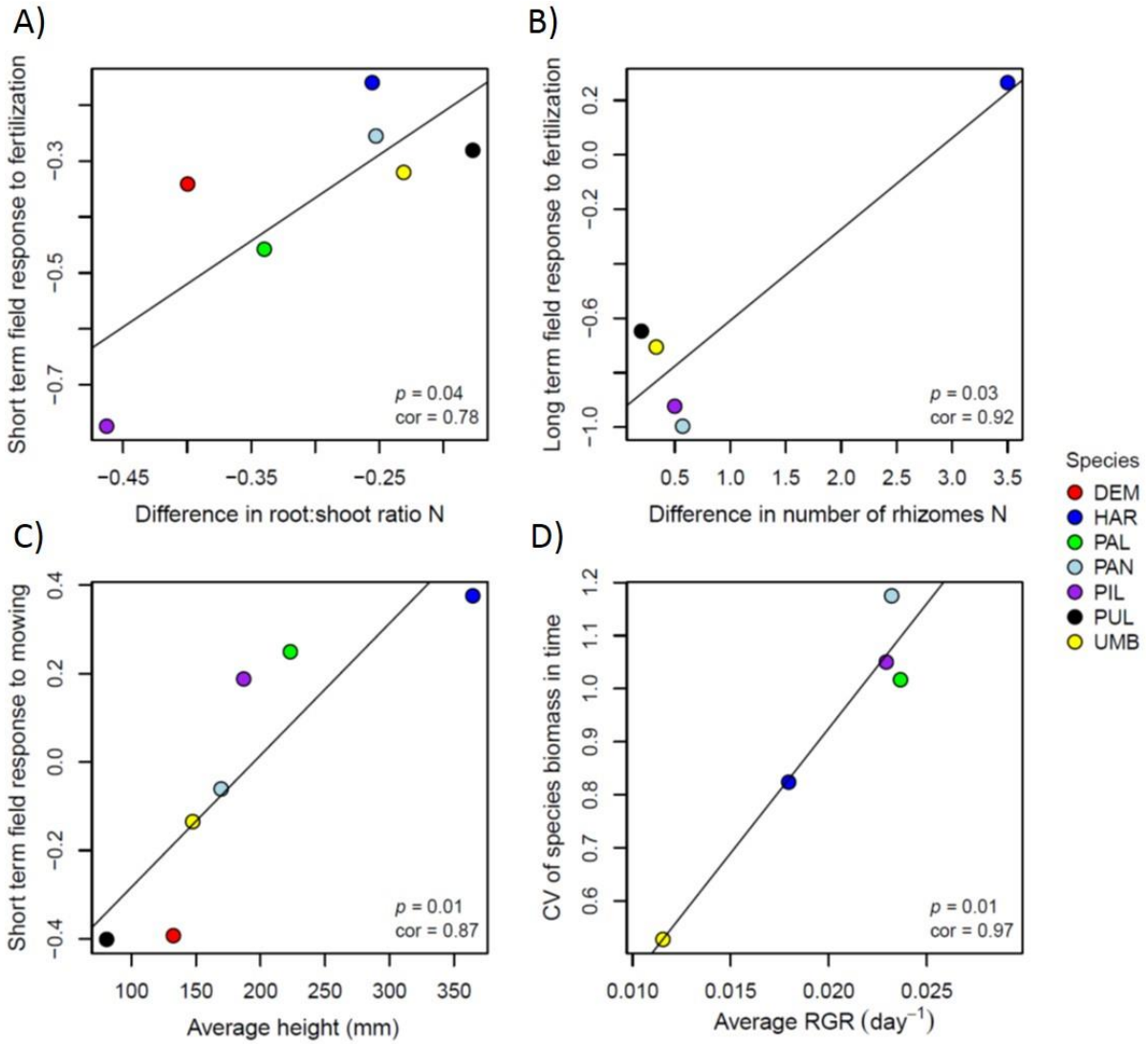

Figure 4. Relating results from the field to results of pot experiments. Lines represent linear regression, p-values of these and Pearson correlation coefficients are marked for each subfigure. (A) Short-term field response to fertilization explained by the difference in root:shoot ratio in fertilization pot experiments. (B) Long-term field response to fertilization explained by the difference in number of rhizomes in fertilization pot experiments. Note, this correlation is mainly driven by one species *C. hartmanii*. *C. demissa* and *C. pallescens* were excluded from the model because they do not produce rhizomes. (C) Short-term field response to mowing explained by the average height. (D) CV from the field explained by the average RGR. *C. demissa* and *C. pulicaris* were not included because there are no reliable estimates of CV due to species rarity. (DEM – *C. demissa*, HAR – *C. hartmanii*, PAL – *C. pallescens*, PAN – *C. panicea*, PIL – *C. pilulifera*, PUL – *C. pulicaris*, UMB – *C. umbrosa*)

3.2. Predicting field responses

The best predictor of short-term field response to fertilization was the difference in root:shoot ratio in response to fertilization from the pot experiment, while the best predictor for long-term response was the difference in number of rhizomes (Appendix 4, Figure 4A and 4B respectively). The best predictors of field responses to mowing were the average height in the short-term (Appendix 4, Figure 4C) and the difference in number of ramets in the long-term (although not significant; Appendix 4). Temporal variability of the biomass of species in the field was best predicted by the RGR value of that species (Appendix 4, Figure 4D). It should be noted that, even though the best predictor was selected in each case, very few significant relationships were found.

4. Discussion

To explain the coexistence of several *Carex* species, we expected variation in species responses to nutrient availability and competition. Variable resource use would offer an explanation for this level of coexistence through niche differentiation, as predicted by limiting similarity (MacArthur & Levins 1967). However, we observed only a few instances where species responses to nutrient availability and competition varied significantly. In contrast to our expectation, we found similar species responses to the treatments with a lack of significant interactions between species and treatments (Table 1). The growth of all the species was negatively affected by competition, and the response to increased soil nutrients was weak for all species (except the root:shoot ratio, and some effect on RGR). Despite a considerable addition of nutrients, biomass only increased weakly. In the pot experiment, a 1 g dose of fertilizer (medium level) in a 16 cm diameter pot matched the dose used in the field experiment (ca 50 g of fertilizer per m^2), in line with standard meadow fertilization regimes (i.e. 95 kg of N per ha, based on the 19% of N of the fertilizer used). This result agrees with Lepš (1999) where *Carex* species did not increase in biomass following field fertilization, despite increasing total community biomass. Across our focal *Carex* species, only the clonal traits showed some differential responses to competition (rhizomes, Table 1B; production of ramets, interaction species × competition × time, Table 2), supporting the view that the response of clonal traits and their temporal variation diverge among *Carex* species in their reaction to competition. The results also show marked differences between species traits, in both pot and field experiments. The differences were particularly clear

in RGR and clonal growth characteristics. Despite these differences, their predictive power for species performance in the field experiment remained weak.

4.1. Fertilization

The effect of a decreased root:shoot ratio by fertilization was shared across all species. Fertilization also seems to have some positive effect on RGR, however, the number of ramets, height, total dry weight, and number of rhizomes were not affected. Numerous other studies also show that increasing fertilization often has the effect of decreasing the root:shoot ratio in various species (for example: Aerts et al. 1992 where all *Carex* species responded similarly, with no species-fertilization interactions; and Li et al. 2010). An increase in nutrients generally promotes growth of aboveground photosynthetic tissue at the expense of root growth. Aerts et al. (1992) also demonstrated that “high-productive species” profited the most from increased nitrogen levels and increased their biomass production with lower root:shoot ratios, in contrast to “low-productive species”.

According to Bernard et al. (1988), *Carex* species are typically more capable of nutrient uptake when availability is low, an idea reinforced by the findings of our experiments. The *Carex* species concerned in this study are mostly confined to low nutrient habitats (Řepka & Grulich 2014) with no species responding positively to fertilization in our field experiment. This probably explains why, in the pot experiment, increased nutrient availability gave little advantage, even when additional resources promoted above ground investment (indicated by lower root:shoot ratios). No significant difference in the response of *Carex* to nutrient availability was detected, thus, it seems unlikely that possible differences in soil-nutrient acquisition can facilitate *Carex* coexistence.

4.2. Competition

Where the response to nutrients was generally very weak, all species reacted significantly to competition, which caused a more marked response than fertilization. The presence of the competitor species *Holcus lanatus* significantly decreased RGR,

546 the number of ramets, and the total dry weight. As well as competing for light, we
547 assume *H. lanatus* also competed for below ground resources with our *Carex* species.
548 As with fertilization, these responses were ubiquitous and, with the exception of clonal
549 traits, showed a lack of significant species × competition interactions. We found
550 significant variation in species where the number of rhizomes changed in response to
551 competition. This corresponded to field results, where *C. hartmanii* produced long
552 rhizomes (see Appendix 1), representing a typical 'guerilla' type growth strategy. In pot
553 experiment, *C. hartmanii* produced most rhizomes at low competition level. With the
554 ability to move using underground rhizomes, *C. hartmanii* is probably able to avoid
555 competition pressure. Thus, variability in the abilities of *Carex* species to respond to
556 competition through different clonal growth strategies, may represent an important
557 mechanism enabling the coexisting of species through circumventing the forces of
558 competitive exclusion (Klimešová et al. 2018).
559 Similarly, the number of ramets over time also changed in response to
560 competition (Table 2 and Figure 3). Indeed, this response had the clearest variation
561 across species (significant triple interaction of species × competition × time, Table 2),
562 in species with different clonal growth responses, including those with different
563 temporal responses. This suggests it is not the differences in traits that characterize
564 productivity, but rather the clonal characteristics that responded differently to
565 competition. We also observed pronounced differences between individual species in
566 their architecture of rhizome systems and thus ability to spread laterally in the field (see
567 Appendix 1). Therefore, we can expect differences in clonal traits and their response
568 to competition to cause differences in spatial foraging for nutrients, which might also
569 contribute to the coexistence of otherwise similar species (He et al. 2007; Klimešová
570 et al. 2018). Vojtko et al. (2017) also suggest that clonal traits are a significant factor
571 enabling the coexistence of similar species, calling for further investigation into the
572 significance that clonal traits play in overcoming competitive barriers.
573 That *Carex* species reacted considerably to competition in the pot experiment,
574 yet community composition did not respond to the removal of the dominant species in
575 the field experiment (i.e. decrease of competition pressure), is not inconsistent. In pot
576 experiments, individuals either were, or were not under competition pressure. In the
577 field experiment, although the removal of *Molinia* would have provided some direct
578 relief from competition with this single species, any gains would have been negated by
579 increased competition with the remaining species in the community. Generally, after

removal, all the species struggled to occupy the new space, so the effect on individual species was not so pronounced (Lepš 1999, 2014). Moreover, all *Carex* species were suppressed by *Holcus*, suggesting they were themselves relatively weak competitors, making it likely that other competitors in the removal plots would suppress them.

4.3. Predictions of field experiment responses

Generally, only weak relationships between our pot and field experiments were detected. In this context, it is important to stress that even when we adopted a rather liberal approach to predicting field responses by pot experiments (see methods), the selection resulted in very weak predictions. No significant predictors were detected for the long-term response to mowing. For the short-term response to fertilization, the strongest relationship was also rather weak (p = 0.036) and for the long-term field response to fertilization, the significant results were likely driven by a single outlier (*C. hartmanii*). These results suggest very weak correspondence of experimental and field data. As the source plants used in these experiments originate from a single population, it is possible that a different population might have responded differently to competition and fertilization because of local adaption process (documented for *Carex* species by He et al. 2007, Schmidt et al. 2018). In our case, however, the experimental plants for the pot experiment were taken from the locality of the field experiment, so that both should have the same local adaptations.

The incongruence between results from the pot and field experiments might also be caused by the combined interactions with other species in the field (absent in the pot experiments). Moreover, there was little variation in the response of *Carex* species in the pot experiments. Where differences were apparent, they were mostly in underground clonal traits. Despite a recent increase in studies promoting the importance of clonality to niche segregation and the coexistence of species (for example: de Bello et al. 2011; Klimešová et al. 2016), such traits are rarely studied, due to the difficulty of measuring them. In this context, the difference in number of rhizomes in the high and no fertilization sets was the best predictor of the long-term field response to fertilization, although this evidence was mainly driven by one species (*C. hartmanii*, Figure 4B).

The short-term response to mowing was best predicted by plant height, with taller plants showing more positive response than shorter species. This goes against our expectation that taller species would be affected more negatively by mowing (Noy-Meir et al. 1989, Opdekamp et al. 2012). At a constant mowing height, a larger proportion of the aboveground biomass would be removed. In addition, the height advantage in competition for light is also removed. However, Klimešová et al. (2008) noted that plant height, often considered the best predictor of a species' response to grassland management, is often coupled with other more relevant functional traits. Within our species, this relationship was driven mainly by the tall *C. hartmanii* which also has the most extensive rhizome system – the most distant connected ramets in the field were more than one meter apart from each other (see Appendix 1). This might explain how *C. harmanii* is able to respond positively to mowing. Furthermore, the correlation was only positive in the short-term, suggesting *C. hartmanii* can quickly recover from mowing while resources are not limited. However, over longer time scales this response would likely change, as below ground resources are gradually depleted. For this reason, we presume an indirect relationship between height and response. *C. hartmanii* can accumulate large belowground nutrient stores in their rhizomes, which can readily be mobilized after mowing. This also illustrates the limitation of the pot experiments, where the potential of this species for clonal spreading could not be demonstrated.

Species temporal stability in the field experiment, characterized by temporal variation in biomass (Harrison 1979; Májeková et al. 2014), was well predicted using RGR from pot experiments. Previous studies suggest that slow-growing long-lived species have more stable biomasses over time because of their reduced responsiveness to environmental change (for example Lepš et al. 1982). These patterns are usually assessed using indirect proxies such as traits linked to the leaf-economy spectrum. For example, Májeková et al. (2014) demonstrated that CV is negatively correlated with LDMC. The theory of r-K strategy (Pianka 1970) also predicts that r-selected organisms will exhibit more pronounced abundance fluctuations in time, because of their higher population level growth rate (Southwood et al. 1974). However, this relationship has so far only been demonstrated in insects (Spitzer et al. 1984) and to the best of our knowledge, the relationship between RGR and population fluctuation has not been demonstrated in plants.

In clonal plants, population growth rate is difficult to measure because of the challenge of identifying individuals. Consequently, we have used RGR based on the biomass changes in potted plants. This is probably a fair proxy for population growth rate, and, in this case, predicted temporal variability in biomass in the field. This agrees with the findings of Májeková et al. (2014), obtained using LDMC, which is expected to negatively correlate with growth rate.

Our results suggest the main differences among the studied *Carex* species were in their clonal traits, in particular, the size of rhizome networks. The *Carex* species in this study generally have rather conservative growth strategies. However, their clonal performance proved to be highly diverse. Species, such as *C. hartmanii*, possess extensive rhizome systems that correspond to typical guerilla strategies, while others, such as *C. umbrosa*, grow in tussocks. Our study clearly shows that the main differences among these closely related species are in clonal traits (and their responses) and that the clonal behavior of our focal *Carex* species is highly variable. This variation in clonal responses and strategies is likely to allowing them to escape competitive exclusion, thus enabling the coexistence of these closely related *Carex* species.

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Data availability

The data for this project are provided as Appendix 5.

Author contributions

Keily Tammaru: Writing – Original Draft, Writing – Review & Editing; **Jan Košnar**: Investigation, Writing – Review & Editing; **Amira Fatime Abbas**: Formal anlaysis, Writing – Review & Editing, Visualization; **Karola Anna Barta**: Formal anlaysis, Writing – Review and Editing, Visualization; **Francesco de Bello**: Writing – Review and Editing, Visualization; **Stefan Harrison**: Writing – Review and Editing; **Emilia Innocenti Degli**: Formal anlaysis, Writing – Review and Editing, Visualization; **Réka Kiss**: Formal anlaysis, Writing – Review and Editing, Visualization; **Katalin Lukács**: Formal anlaysis, Writing – Review and Editing, Visualization; **Szilvia Márta Neumann**: Formal anlaysis, Writing – Review & Editing, Visualization; **Hayden Wagia**: Writing – Review & Editing; **Javier Puy**: Conceptualization, Methodology, Formal analysis, Visualization, Writing – Review & Editing, Supervision; **Jan Lepš**: Conceptualization, Methodology, Writing – Review & Editing, Supervision.

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Table 1. Influence of species and fertilization (A) and competition (B) level on six traits, tested by two-way ANOVA. For testing the effect on the number of rhizomes only four species could be used in the analysis. The bold numbers indicate significant effects ($p < 0.05$). For the fertilization experiment Error df = 65, except for number of rhizomes where Error df = 46. For the competition experiment Error df = 84, except for height and root:shoot ratio where Error df = 77,and number of rhizomes where Error df = 48.

A	df	RGR		Number of ramets		Height		Total dry weight		Root:shoot ratio		df	Number of rhizomes	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*		*F*	*p*
Species	6	9.687	**<0.001**	8.429	**<0.001**	50.086	**<0.001**	17.922	**<0.001**	5.029	**<0.001**	4	6.957	**<0.001**
Nutrients	2	3.088	0.052	1.365	0.263	0.602	0.551	1.675	0.195	51.015	**<0.001**	2	0.455	0.637
Species:Nutrients	12	0.891	0.559	1.137	0.347	0.830	0.619	0.783	0.666	0.787	0.662	8	0.171	0.994

B	df	RGR		Number of ramets		Height		Total dry weight		Root:shoot ratio		df	Number of rhizomes	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*		*F*	*p*
Species	6	18.215	**< 0.001**	8.487	**< 0.001**	23.846	**< 0.001**	23.502	**< 0.001**	4.845	**< 0.001**	3	22.281	**< 0.001**
Competition	2	21.821	**< 0.001**	27.547	**< 0.001**	0.336	0.715	14.125	**< 0.001**	0.158	0.854	2	3.171	0.051
Species:Competition	12	0.637	0.805	1.839	0.055	0.959	0.494	0.924	0.527	0.799	0.650	6	3.346	**<0.008**

Supplementary materials

Appendix 1. Rhizome systems of individual *Carex* species as uncovered in the field.

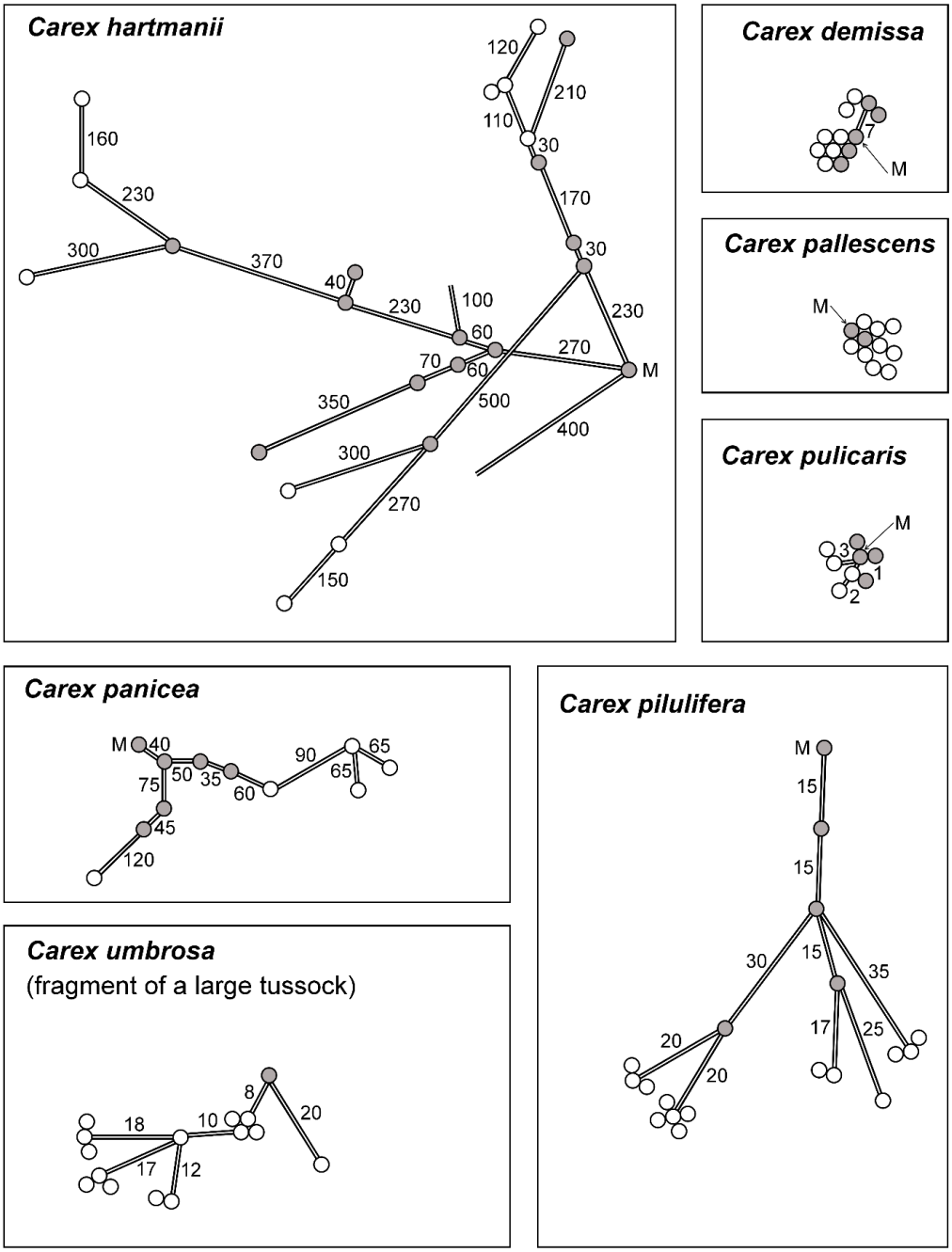

Figure S1. Rhizome systems of individual *Carex* species as uncovered in the field. All the depicted plants came from the locality Ohrazení. Their rhizome systems were uncovered in the second half of October 2001 (*Carex hartmanii*) and 2002 (the other species). *Symbols:* empty circle – ramet with living leaves; full circle – ramet without living leaves; double lines – rhizome branches; M – maternal ramet; numbers – length (mm). *Note 1:* Rhizome systems of *Carex pilulifera*, *C. demissa*, *C. pallescens*, *C. pulicaris* and *C. umbrosa* are shown at 10-times greater scale than those of *C. hartmanii* and *C. panicea*. *Note 2:* Rhizome branches of *C. umbrosa* are rather ascending than horizontal. Thus, the distances between ramets in the field are somewhat smaller than depicted.

Appendix 2. Analysis of model validity

The validity of each model presented in the manuscript was tested, for example checking the normality and homoscedasticity of the residuals. In the following figures we show the distribution of the residuals of each of the models: Figure S2 – univariate analysis of variance (ANOVA) of several traits in fertilization experiment; Figure S3 – ANOVA of several traits in competition experiment; Figure S4 – linear regression between experiment and field responses. For the latter, note the low N of the model.

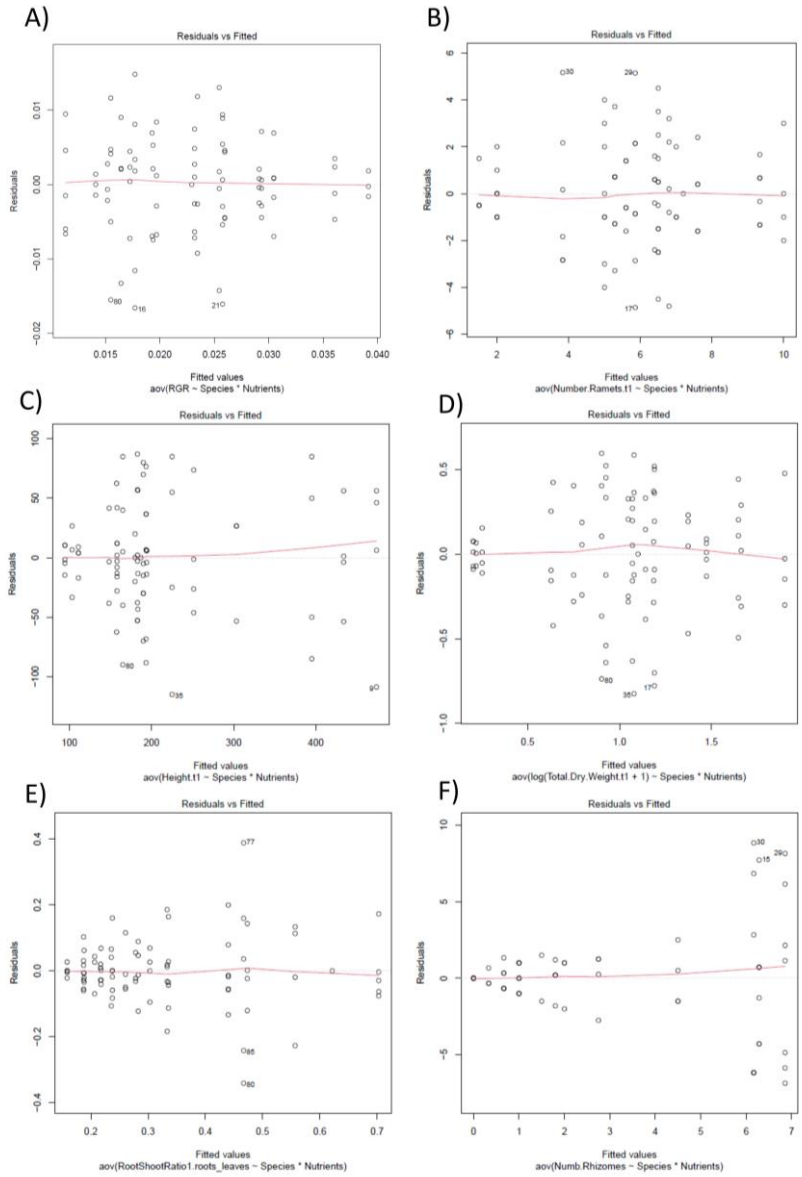

Figure S2. Distribution of the residuals of the ANOVA models for each particular trait from the fertilization experiment. A) Relative growth rate, B) Number of ramets, C) Height, D) Total dry weight in logarithmic scale, E) Root:shoot ratio and F) Number of rhizomes.

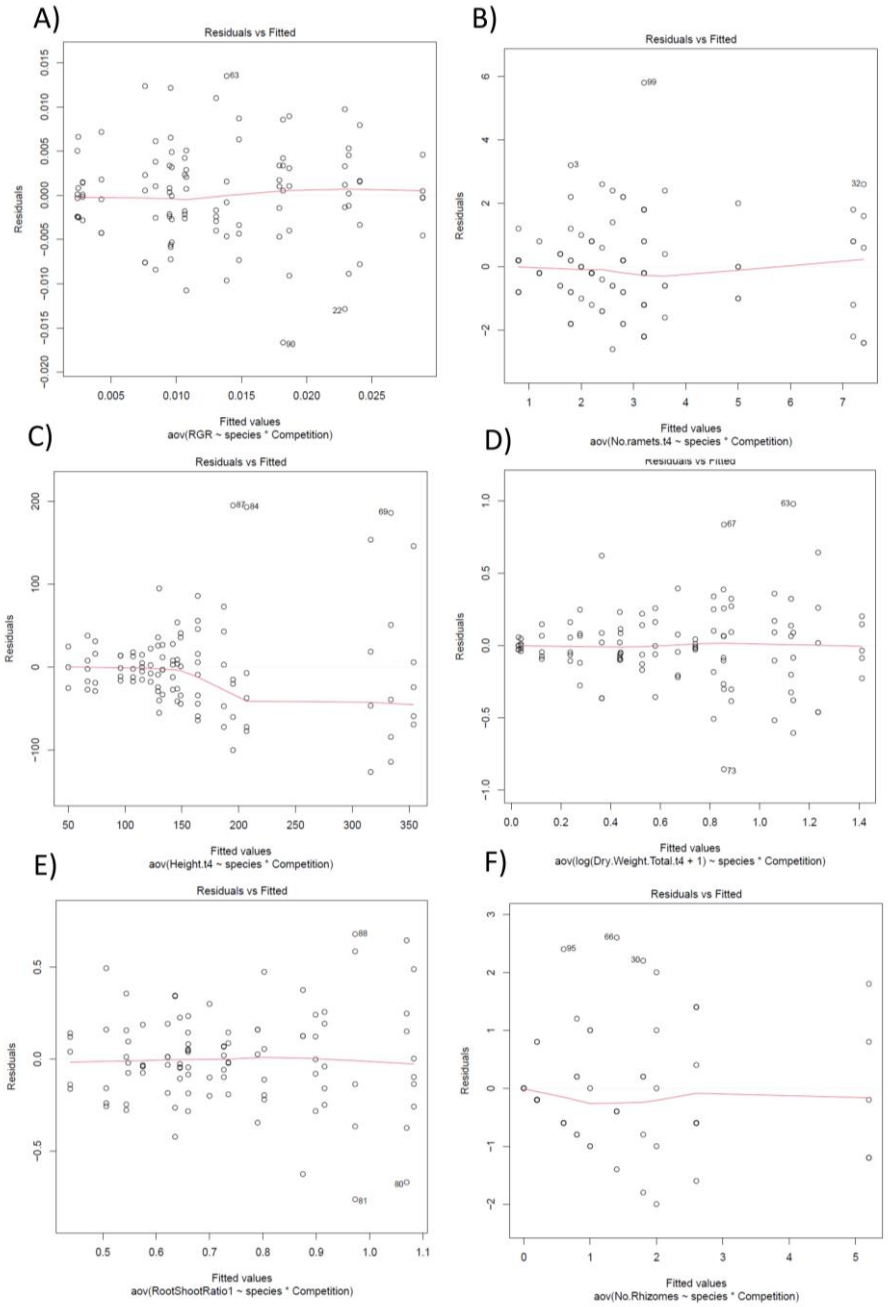

Figure S3. Distribution of the residuals of the ANOVA models for each particular trait from the competition experiment. A) Relative growth rate, B) Number of ramets, C) Height, D) Total dry weight in logarithmic scale, E) Root:shoot ratio and F) Number of rhizomes.

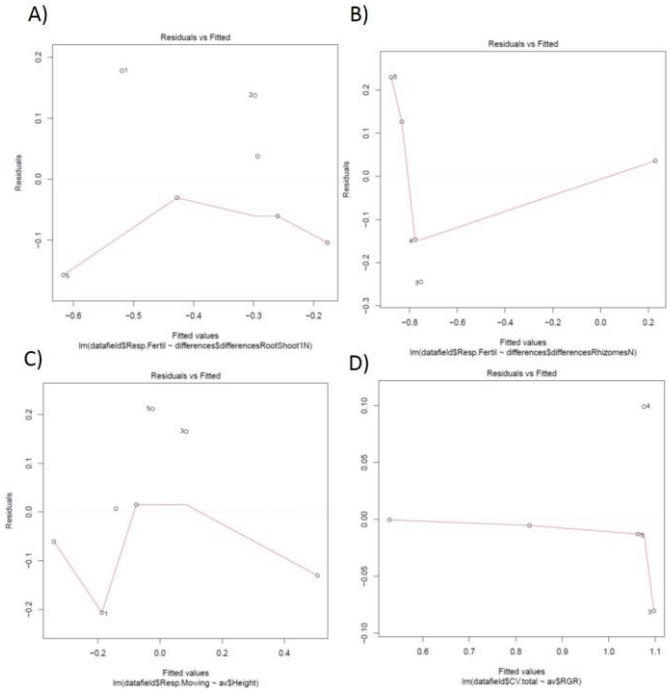

Figure S4. Distribution of the residuals of the several linear models used for the predictions of the field response by the response from the pot experiments. A) Short-term field response to fertilization explained by the difference in root:shoot ratio in fertilization pot experiments. B) Long-term field response to fertilization explained by the difference in number of rhizomes in fertilization pot experiments. C) Short-term field response to mowing explained by the average height. D) CV from the field explained by the average RGR.

Appendix 3. Multivariate analysis of the two experiments

In both experiments, we used the Redundancy Analysis (RDA; Šmilauer and Lepš 2014), with the five characteristics available for all the species as response variables (i.e. relative growth rate (RGR), root:shoot ratio, height, number of ramets, log of total dry weight), and species and treatment (i.e. either nutrients, or competition level) as predictors (both considered factors, i.e. the categorical variable). We have not used the number of rhizomes, because not all the species formed rhizomes in the experiment. The analyses were designed to correspond as much as possible to the ANOVA for the univariate response. The tests of the main effects (i.e. either treatment or species) were obtained from partial RDA, with the effect tested being the explanatory variable, and the other the covariable. The test of the interaction (treatment × species) was obtained by partial RDA, with the interaction being the explanatory variable, and both the main effects being the covariables. All the analyses provided amount of explained variability and pseudo-F statistics, which was used to test the significance by the Monte Carlo permutation test with 4999 permutations. Note that the amount of explained variability is dependent on degrees of freedom, which is quite different – for treatment, df = 2, for species, df = 6. Amount of explained variability is provided as percentage of the total variability in the response variables. The ordination diagrams also provide a lead on the correlation of individual response variables.

For the competition experiment, for few individuals, some characteristics were not available (for the individuals that died during the experiment, we were not able to provide root:shoot ratio, together × individuals). Because RDA needs complete samples, we calculated two versions, first with the complete cases only, and then with the missing values replaced by the mean of the variable. The two versions provided nearly identical results, and we present here the one with substitution of the mean. Results are summarized in Table S1.

Table S1. Summary of the results of the partial RDA with the main effects of treatment and species for the fertilization experiment and competition experiment.

Fertilization experiment	df	Explained variability	pseudo-F	*p*
Species	6	46.3	14.2	0.0002
Fertilization	2	11.6	10.6	0.0002
Species × Competition	12	6.1	0.91	0.5786

Competition experiment	df	Explained variability	pseudo-F	*p*
Species	6	40.4	13.3	0.0002
Competition	2	11.0	10.9	0.0002
Species × Competition	12	6.4	1.07	0.3654

The results show that the differences among species are still more pronounced than are differences between treatment levels: the species factor uses 6 df vs 2 df for the treatment, whereas the explained variability by species is more than three times higher, and usually, the explained variability increases with the df less than linearly, so the interspecific differences are more pronounced than the differences among treatment levels. In both experiments, the treatment × species interaction is not significant, and explains negligible amount of variability.

Further, we provide the ordination diagrams characterizing the effect of the treatments, i.e. the RDA with treatment as the explanatory, and species as covariable.

Both the ordination diagrams show significant effect of the treatment levels and the response variables. The ordination diagram for fertilization (Figure S5) shows that the root:shoot ratio is the most responsive characteristics to fertilization, with highest values in non-fertilized plots, and that weight, RGR, number of ramets are positively affected by (mainly high) fertilization.

The ordination diagram for competition (Figure S6) also shows that the main difference is between the no competition level and the competition (either low or high). Note the pronounced difference between variability explained by the first and the second axis, ascertaining that the truly different level is the no competition. It also shows that the plants without competition have higher weight, RGR, number of ramets and these three are highly correlated. The height and root:shoot ratio are not affected, which perfectly corresponds with the univariate analyses.

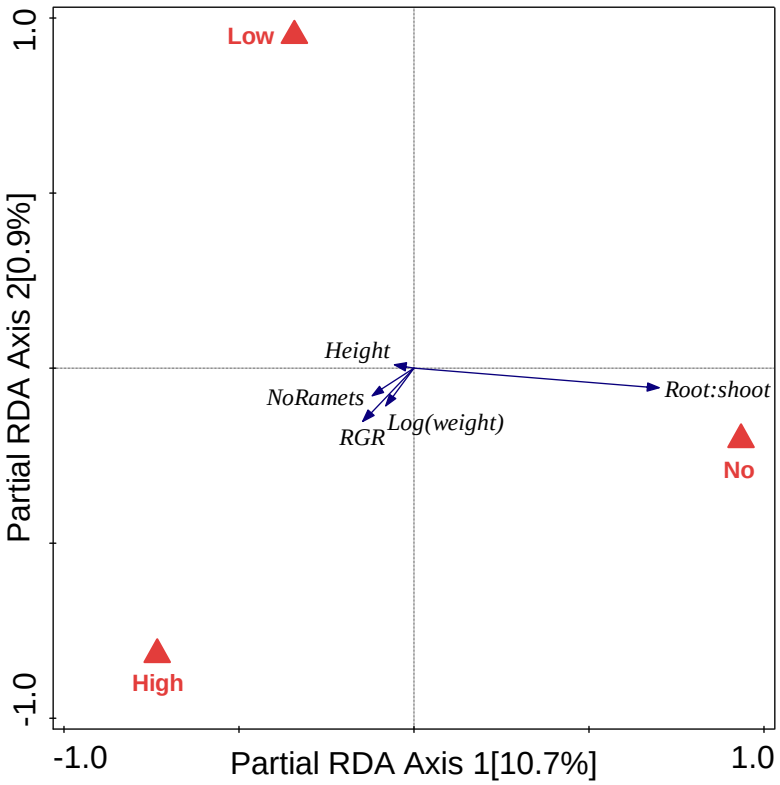

Figure S5. Effect of levels of fertilization (shown by red triangles, as centroids) on the characteristics of individuals. The values in axes labels brackets signify percentage of the total variability of the response variables explained by given axis.

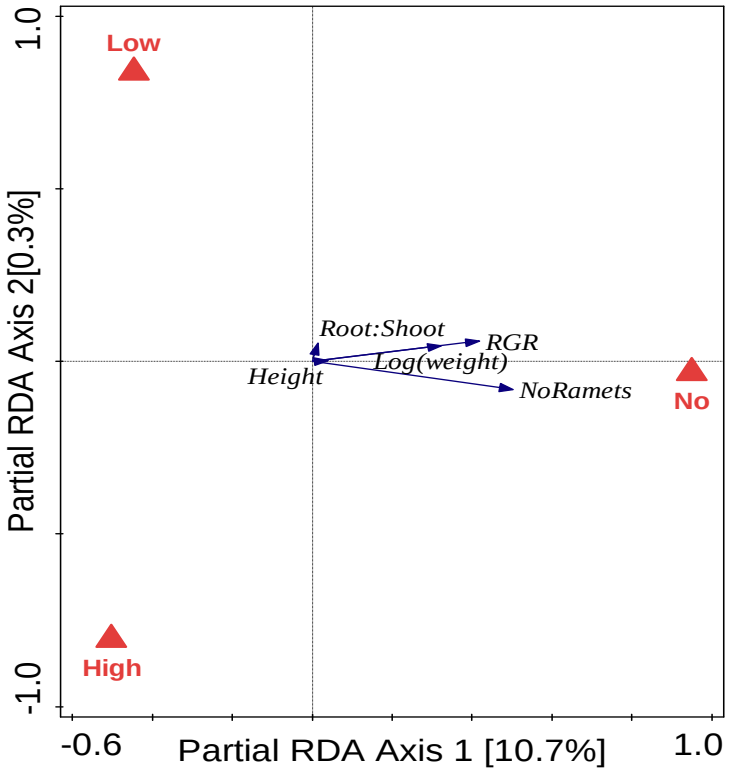

Figure S6. Effect of levels of competition (shown by red triangles, as centroids) on the characteristics of individuals. The values in axes labels brackets signify percentage of the total variability of the response variables explained by given axis.

In summary, the results of the multivariate analyses of both experiments show that the treatment has an effect on the characteristics measured and thus the significant results for individual characteristics are not solely effect of Type I error (they are not likely to be just a consequence of “statistical fishing”). Expectedly, the RDA have shown the most responsive characteristics, and these are those with the significant main effect. Nevertheless, in concert with the univariate analyses, the species × treatment interactions are not significant (both with $p > 0.3$).

Reference

Šmilauer, P., Lepš, J. 2014. *Multivariate analysis of ecological data using Canoco 5*. Cambridge university press.

Appendix 4. Predicting short and long-term responses and temporal variability of the species in the field.

Table S2. Predicting short and long-term responses and temporal variability of the species in the field. Results of the analysis of best explanatory variables for the field responses. Best predictors are selected from the response variables of the pot experiments. (N – fertilization pot experiment, C – competition pot experiment, R^2_{Adj} – Adjusted R-squared, df – residuals degrees of freedom)

Field responses	**Best predictor**	**Standardized coefficient**	**R^2_{Adj}**	***F***	***df***	***p***
Short-term field response to fertilization	differences root:Shoot N	0.78	0.54	7.97	5	0.037
Long-term field response to fertilization	differences rhizomes N	0.92	0.80	17.34	3	0.025
Short-term field response to mowing	average Height	0.87	0.72	16.08	5	0.010
Long-term field response to mowing	differences ramets C	-0.67	0.31	3.24	4	0.146
Coefficient of variation	average RGR	0.97	0.91	43.42	3	0.007