Belowground competition drives invasive plant impact on native species regardless of nitrogen availability

Arthur A.D. Broadbent[1*], Carly J. Stevens[1], Duane A. Peltzer[2], Nicholas J. Ostle[1] and Kate H. Orwin[2]

1 - Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YW, UK

2 - Landcare Research, PO Box 69040, Lincoln 7640, New Zealand

*Corresponding author, orchid id: orcid.org/0000-0002-8438-7163;

Email: a.broadbent2@lancaster.ac.uk

Tel: +44 (0)7549 689932

Author contributions: AB and CS conceived the experiment; AB conducted the experiment and data analysis; DP coordinated fieldwork; AB, CS, DP, NO and KO designed experiments and wrote the manuscript.

Abstract

Plant invasions and eutrophication are pervasive drivers of global change that cause biodiversity loss. Yet, how invasive plant impacts on native species, and the mechanisms underpinning these impacts, vary in relation to increasing nitrogen (N) availability remains unclear. Competition is often invoked as a likely mechanism, but the relative importance of the above and belowground components of this is poorly understood, particularly under differing levels of N availability. To help resolve these issues, we quantified the impact of a globally invasive grass species, *Agrostis capillaris*, on two co-occurring native New Zealand grasses, and vice versa. We explicitly separated above and belowground interactions amongst these species experimentally and incorporated an N addition treatment. We found that competition with the invader had large negative impacts on native species growth (biomass decreased by half), resource capture (total N content decreased by up to 75%) and even nutrient stoichiometry (native species tissue C: N ratios increased). Surprisingly, these impacts were driven directly and indirectly by belowground competition, regardless of N availability. Higher root biomass likely enhanced the invasive grass's competitive superiority belowground, indicating that root traits may be useful tools for understanding invasive plant impacts. Our study shows that belowground competition can be more important in driving invasive plant impacts than aboveground competition in both low and high fertility ecosystems, including those experiencing N enrichment due to global change. This can help to improve predictions of how two key drivers of global change, plant species invasions and eutrophication, impact native species diversity.

Keywords

Global change, grassland, mechanism, non-native, nutrient availability.

Introduction

The rapid increases in plant species invasions and soil nitrogen (N) availability are major drivers of global change and biodiversity loss (Vitousek et al. 1997; Stevens et al. 2004; Vilà et al. 2011; Seabloom et al. 2015; Van Kleunen et al. 2015). However, how increasing N availability alters invasive species impacts on native species, and the mechanisms underlying those impacts, remains uncertain. A stronger mechanistic understanding of invasive plant impacts under varying edaphic conditions would enable better prediction of where and when impacts on native species are most likely to occur. In turn, this could facilitate pre-emptive management to prevent negative impacts, thereby protecting native species diversity and ultimately, reducing global biodiversity loss.

Interspecific competitive interactions are often proposed as the primary pathway for invasive plant impacts, yet few studies experimentally test whether, or which, competitive mechanisms underlie invader impacts (Levine et al. 2003; Seabloom et al. 2003; Tylianakis et al. 2008; Barney et al. 2013, 2015). For example, the relative importance of above and belowground competitive interactions in determining invasive plant species impacts is unclear. Further uncertainty arises from the likely shifts in importance of different impact mechanisms, such as above and belowground competition, following increases in soil nutrient availability. We found only two experiments that explicitly separated above and belowground competitive interactions between native and invasive species and both reported that belowground competition was more important in delivering invader impacts (Dillenburg et al. 1993; Kueffer et al. 2007). However, other lines of evidence suggest aboveground competition may also be a strong driver, particularly following increases in nutrient availability (Tilman 1982; Cahill 1999; Hautier et al. 2009; Borer et al. 2014). Indeed, increased soil fertility can increase the competitive superiority of invasive plants (Daehler 2003; Besaw et al. 2011), which tend to have more exploitative traits than co-occurring native species (Leishman et al. 2007; van Kleunen et al. 2010; Ordonez et al. 2010). These traits should be advantageous under high resource conditions and in competing for light, although evidence for this is mixed (Leishman et al. 2010; Ordonez and Olff 2013). Furthermore, the invasive plant species with the most exploitative aboveground traits tend to have the greatest negative impact on native species (Lai et al. 2015). It is therefore likely that both above and belowground competition can underpin invasive species impacts and that it depends on belowground resource availability, yet experiments that test this explicitly remain remarkably rare.

Increasing N availability to increase productivity is common practice in grasslands, where invasion rates are among the highest worldwide (Firn et al. 2011). Additionally, co-occurring invasive and native grass species are often closely related and functionally similar. Grasslands thus constitute ideal model systems in which to test the importance of above and belowground competition as mechanisms of invasive plant impacts across varying levels of soil N availability. One such system is low-fertility New Zealand grassland that is designated as valuable conservation habitat (Mark and McLennan 2005; Rose and Frampton 2007). These grasslands are experiencing widespread declines in native species diversity (Duncan et al. 2001) associated with non-native grass invasions (Rose et al. 2004) and increases in nitrogen (N) availability (Scott 2000; Dickie et al. 2014). Invasive grasses in New Zealand, and elsewhere, tend to have more exploitative traits than co-occurring native grasses (Craine and Lee 2003; Wilsey and Polley 2006), suggesting that invasive grasses may be superior aboveground competitors compared to native grasses (Johnson et al. 2008; Lai et al. 2015). Their aboveground competitive superiority is likely to increase following increases in soil N availability, as this would lead to N no longer being a limiting resource, thereby allowing competition to shift aboveground for light (Wilson and Tilman 1991; Aerts 1999), as has been observed in various grasslands worldwide (Tilman 1988; Bobbink 1991; Hautier et al.

2009). However, as far as we are aware, there are no experiments that test this idea by explicitly separating above and belowground competition across varying levels of N. To address this, we test the following specific hypotheses:

1. Competition between invasive and native grass species benefits the invasive species, *Agrostis capillaris* L., and decreases native species, *Poa cita* Edgar and *Poa colensoi* Hook.f., growth and resource capture, compared with intraspecific competition;
2. *A. capillaris'* competitive impacts on native species, and the relative importance of aboveground competition over belowground competition, both increase with increasing N availability.

Methods

EXPERIMENTAL DESIGN AND GREENHOUSE CONDITIONS

We determined the effects of above- and belowground competition of a globally invasive grass, *A. capillaris*, on two common perennial C_3 tussock grasses native to New Zealand: *P. cita* and *P. colensoi*. *A. capillaris* is a Eurasian rhizomatous perennial C_3 grass species (height = 20-70 cm) that is one of the most widespread invasive grasses in New Zealand (Edgar and Forde 1991; Craine and Lee 2003; CABI 2017) and is also a pervasive weed in North America, Australia and parts of South America (CABI 2017). It is a habitat generalist that occurs across a wide range of climatic and edaphic conditions in its native and introduced ranges (CABI 2017). These include Atlantic and continental climates at low and high altitudes, along with low fertility, usually acidic, soils, as well as nutrient rich meadows (Hill et al. 1999; Olde Venterink and Güsewell 2010; CABI 2017). *P. colensoi* (height = 5-30 cm) and *P. cita* (height = 30-100 cm) are both short tussock grass species, widespread throughout

New Zealand, usually on low fertility acidic soils in montane areas (Daly 1964; Edgar and Connor 2000). Our species were con-familiar and from the same functional group, which controlled for confounding effects due to lifeform (Vila and Weiner 2004). Seeds of all species were sourced from NZ populations by Speciality Seeds and Home Creek Nursery.

Treatments consisted of a full factorial cross of two native species identities (*P. cita* and *P. colensoi*), four competition types (*control or full intraspecific competition, aboveground competition, belowground competition and full competition*; Fig. 1) and two N addition treatments (*control or "low N" and N-addition or "high N"*); with five replicates this made a total of 80 pots. For each native - invasive species combination, two individuals of the native species and two individuals of the invasive species were planted into 1 L pots. To minimise lateral escape from competition, pots were surrounded by a 1 mm nylon mesh that was 10 cm high. In order to separate above- and belowground competition, the pots were split using solid PVC dividers within the pot, sealed with PVC glue and silicon, and opaque plastic dividers above the pot. By varying the alignment of these dividers, four competition treatments were created: 1) *control or intraspecific competition*, where plants were competing with conspecifics above- and belowground; 2) *aboveground* and 3) *belowground*, where plants were either competing with heterospecifics aboveground and conspecifics belowground, or vice versa, respectively; and 4) *full competition*, where plants were competing with heterospecifics above- and belowground (Fig. 1). Treatments were placed in a randomised block design and blocks were rotated weekly. Our replacement design thus kept plant density constant both overall and in each component of the pot (Fig. 1). This design compares the effect of intraspecific competition with interspecific competition, which is sometimes criticised due to the lack of a "zero" competition control, where plants are grown without neighbours. However, invaded grassland communities often become relatively space limited in the absence of a disturbance; therefore in field conditions, it is more likely for plants to encounter neighbours. For this reason, our design was arguably more realistic than an additive design, which involves zero competition treatments and inconsistent plant densities.

Nitrogen addition treatment consisted of 133 mg NH_4NO_3 pot^{-1} week^{-1} dissolved in 180 mL de-ionised water, applied evenly over the area of each pot thrice weekly in 60 mL doses. Nitrogen was used to increase resource availability as it is commonly used as an agricultural fertiliser in grasslands. Soil N availability also increases in New Zealand grasslands following invasion and removal of exotic woody species such as C*ytisus scoparius* L. and *Pinus contorta* Douglas (Dickie et al. 2014; Broadbent et al. 2017). This is often followed by exotic grass invasions, including *A. capillaris* (Williams 1998; Dickie et al. 2014), and so might be a mechanism driving invasive success. Our rate of N addition is in line with net soil N-mineralisation rates of grasslands in NZ that have been cleared of invasive N-fixing shrubs (Broadbent et al. 2017).

Pots were filled with a mixture of field soil and autoclaved sand (3:1 by volume) to improve drainage. Field soil was collected (depth = 10 cm) from 40 random locations of grassland-shrubland habitat in St. James Conservation Area in New Zealand (Lat. Long. = -42.460273, 172.830938). Vegetation at the site consisted of a mixture of native and exotic species, including those used in our study. Soil was sieved (4 mm) and homogenised prior to mixing with sand. Mean pot soil pH (1: 2.5, soil: water) was 6.82 ± 0.02 (mean ± one SE), mean KCl extractable N concentration (NO_3^--N and NH_4^+-N) was 2.97 ± 0.15 µg N g soil^{-1} and mean $NaCO_3$ extractable PO_4^--P concentration (Olsen-P) was 4.95 ± 0.29 µg P g soil^{-1}. The soils in our study had low inorganic N concentrations (2.97 ± 0.15 µg N g soil^{-1}) and low N: P ratios (0.6). These concentrations were determined colorimetrically in a segmented flow stream using an AutoAnalyser (Seal-Analytical).

Plants were germinated in potting compost under the same standardised climatic conditions that were used throughout the experiment: lighting regime: Light: Dark 16h: 8h, Temp maximum: minimum 22 ºC: 16 ºC. After germination, seedlings were carefully transferred into plugs, then two weeks later into pots, on the 21st June 2016. The mean mass of seedlings did not differ between species prior to transplanting into pots ($F = 2.1$, $p = 0.13$, one-way ANOVA). This was determined by harvesting, drying (65 ºC for 48 hours) and weighing (± 0.0001 g) the above- and belowground biomass of a random subset of 20 seedlings of each species at the start of the experiment.

Plants were watered equally each day with ca. 100 mL of tap water per pot. The experiment lasted 11 weeks, with all biomass harvested on the 6th September 2016. Biomass from each pot was separated by species and dried at 65 ºC for 48 hours after all soil had been washed from roots. Roots of individuals that were competing belowground were carefully separated. Due to the difficulty of separating roots between species, this was only done for one side of the division in each pot; the biomass value obtained was doubled to give an estimate per species per pot. Biomass was separated into above and belowground components, with aboveground components further separated into live and dead biomass, before being weighed to 0.0001 g.

MEASUREMENTS AND CALCULATIONS

After weighing biomass, belowground and aboveground plant C and N concentrations were measured on ground samples using an automated Dumas procedure on a Vario EL analyser (Elementar). This was used to calculate whole plant mean C: N ratio (the mean of above- and belowground C: N ratios). Since plant tissue C: N ratio is the mass of C relative to the mass of N in plant tissue, it provides a similar measure to tissue %N content, with the advantage of being easily compared to other substrates such as soil. To calculate total N content (g), %N content of above- and belowground biomass components was multiplied by the corresponding biomass (g) and then summed. We also calculated % dead aboveground biomass (of total aboveground biomass) and two allocation patterns: root mass fraction (RMF: belowground biomass/ total biomass) and root nitrogen fraction (RNF: belowground N/ total N).

STATISTICAL ANALYSIS

We determined the effect of competition with invasive species on native species growth and resource capture (hypothesis 1), and whether competition type and N addition modified this effect (hypothesis 2), by using three-way ANOVAs on native species responses. The responses we tested were mean total, aboveground and belowground biomass (g), mean dead aboveground biomass (%), mean total N content (g), mean C: N ratio, mean RMF and mean RNF. Each ANOVA had native species identity (*P. cita* or *P. colensoi*), competition treatment (*control, aboveground, belowground* or *full*), N addition treatment (*low-N* or *high-N*) and all interactions as factors. We also determined the effect of native species competition on invasive species growth and resource capture (hypothesis 1), and whether this differed due to the competition and N addition treatments (hypothesis 2), by using three-way ANOVAs on invasive species responses. We used the same responses and factors in these ANOVAs as for those on native species responses, although the factor "native species identity" now referred to the identity of the native competitor.

If a three-way interaction occurred, then the analysis was split by native species identity/ native competitor identity in order to facilitate interpretation. In this case, two-way ANOVAs were performed on the responses of each native species separately (or the invasive species in competition with each native species separately) with competition treatment, N addition treatment, and their interaction as factors. Tukey HSD pair-wise significant differences (p <

0.05) were determined between all levels of any significant factors, including any interactions. Models that violated assumptions of normality or homoscedasticity received a $\log_{10}(y)$ transformation. All analyses were performed in R version 3.2.4 (R Core Team 2016).

Results

BIOMASS RESPONSES

Native and invasive species growth was impacted in contrasting ways by the competition treatments (Table 1; Fig. 2). Both native species responded similarly to competition with the invader, expect for their belowground biomass (Fig. 2). Compared to the control treatment, belowground competition with the invader reduced native species mean total biomass by a third, whilst full competition reduced it by half ($F = 24.6$, $p < 0.01$, Table 1, Fig. 2a). Conversely, belowground competition with native species increased invasive species mean total biomass by 41% and full competition increased it by 65% ($F = 66.0$, $p < 0.01$, Table 1, Fig. 2a). Belowground and full competition between the native and invasive species increased the mean percentage of dead aboveground biomass on native species by a factor of 4 ($F = 44.7$, $p < 0.01$, Table 2, Fig. S1), while reducing it on invasive species to almost half ($F = 14.4$, $p < 0.01$, Table 2, Fig. S1). Nitrogen addition decreased belowground biomass of native species from 0.47 ± 0.05 g (mean ± one SE) to 0.38 ± 0.04 g and invasive species from 2.20 ± 0.09 g to 1.75 ± 0.08 g. It also increased invader aboveground biomass from 3.14 ± 0.14 g to 3.83 ± 0.18 g. Nonetheless, it did not affect total biomass or dead aboveground biomass, and its effects did not change under the different competition treatments (Tables 1 & 2). Aboveground competition on its own had no effect on native or invasive species growth; however, when combined with belowground competition (i.e. in the full competition treatment) it resulted in a ca. 25% greater decline in native species total biomass, and a greater increase in invasive species biomass, than belowground competition on its own (Fig. 2a). The increase in invasive species total biomass in the full competition treatment was principally driven by increases in aboveground biomass (Fig. 2b), not belowground biomass (Fig. 2c); whereas the decline in native species total biomass came predominantly from a decrease in belowground biomass (Fig. 2d).

RESOURCE CAPTURE AND ALLOCATION RESPONSES

Total N content

Mean total N content (g) of native and invasive species responded in similar but slightly more complex ways to competition than their biomass. The impact of invasive species competition varied by native species identity (F = 2.8, $p < 0.05$, Table 2, Fig. S2) and by N addition (F = 3.5, $p = 0.02$, Table 2, Fig. 3b). *P. cita*'s mean total N content declined by c. 40 and 50 % when competing belowground and fully with the invader (Fig. S2), whereas the mean total N-content of *P. colensoi* decreased by c. 60 and 75 % (Fig. S2), respectively. The negative effect of belowground competition on native species mean total N was almost twice as large under the low N treatment (-63%) than the high N treatment (-34%; Fig. 3b). Similarly, the full competition treatment resulted in a 76% decrease under low N conditions compared with a 55% decrease under high N conditions (Fig. 3b). Invasive species mean total N content increased by over 50% when the invader competed with native species belowground, and by over 75% when they competed fully (F = 79.8, $p < 0.01$, Table 2, Fig 3a), regardless of native species competitor identity and N addition. Aboveground competition had no effect on native or invasive species resource capture on its own; however, when combined with belowground competition (i.e. in the full competition treatment), it resulted in a ~25% greater increase in invasive species mean total N content than just belowground competition on its own (Fig. 3a).

Mean C: N ratio and allocation patterns

There was a three-way interaction between native species identity, competition and N addition treatments on mean C: N ratio of native species (F = 3.3, p = 0.03, Table 2). We therefore split the analysis by native species identity and tested *P. cita* and *P. colensoi* responses separately (Table 3; Figs. 3c-d). The patterns in their responses were relatively similar (Figs. 3c-d). They both showed stable mean C: N ratios across all competition treatments under the high N treatment (Figs. 3c-d). Under the low-N treatment, they both showed increased mean C: N ratios (*P. cita,* +37%; *P. colensoi* +44%) when competing belowground with the invader (Figs. 3c-d). The key difference between the native species was in the control and aboveground competition treatments; where the mean C: N ratio of *P. cita* decreased in the high N treatment, compared to the low N treatment, while *P. colensoi's* mean C: N ratio showed no difference (Figs. 3c-d). The mean C: N ratio of the invasive species, *A. capillaris*, was mostly stable in response to competition treatments, although it was slightly higher in the aboveground than the belowground or full competition treatments (F = 4.0, $p = 0.01$, Table 2; Fig 3e).

The allocation patterns of the invasive species showed no significant variation in response to competition treatments (Table 4; Fig. 3f & S3). However, compared to the control treatment, the native species showed a lower RMF in the full competition treatment (F = 4.7, $p = <0.01$, Table 4; Fig 3f), and a higher RNF in the belowground competition treatment compared to the aboveground competition treatment (F = 3.2, $p = 0.03$, Table 4; Fig S3).

Discussion

Contrary to our expectations that aboveground competition would be more important than belowground competition under elevated N availability, belowground competition was in fact central to the impact of the invasive grass species regardless of N availability. The belowground competitive superiority of the invasive grass, *A. capillaris*, caused large declines in native species biomass and total N content, along with alterations to tissue nutrient stoichiometry and biomass allocation patterns. The invader's impact was greatest under low N conditions but it also remained a superior competitor under high N conditions. Considering the paucity of invader impact studies explicitly separating above and belowground competition, along with a general perception of invasive plants as exploitative species that are likely to be stronger aboveground competitors, we suggest that belowground competition may be an under-appreciated mechanism for invasive plant impacts. This is particularly true in high fertility ecosystems, such as those experiencing N enrichment due to global change.

COMPETITIVE IMPACT

We used direct measures of competition including resource capture, alongside indirect measures such as biomass, in order to determine whether competitive interactions underpinned the differences in species growth (Trinder et al. 2013). Since resource capture rates, i.e. total N content, mirrored the changes in species biomass across competition treatments, it is likely that competitive interactions underpinned these changes. In the full competition treatment, native species' growth and resource capture rates were heavily reduced, while the invader's growth and resource capture increased substantially (Figs. 1 and 2). This supports our first hypothesis that competition between the invasive, *A. capillaris*, and the native grass species, *P. cita* and *P. colensoi*, benefits the invasive species and decreases native species growth and resource capture, compared with intraspecific competition. Competition is therefore a clear pathway for the negative impacts of *A. capillaris* on two co-occurring native grasses. The invader's competitive advantage suggests that fitness differences between the invader and native species are likely contributing to *A. capillaris's* invasive success in these systems (MacDougall et al. 2009). Whilst other invasive plants have been shown to out-compete native species, this often depends on increased nutrient availability (Daehler 2003; Besaw et al. 2011; Seabloom et al. 2015). In contrast, the invader in our study, *A. capillaris*, had large impacts on native species across both high and low N availabilities. This is consistent with previous experiments in native grassland communities, which also found no significant change in competition intensity across soil nutrient gradients (Wilson and Shay 1990; DiTommaso and Aarssen 1991), particularly when interspecific competition intensity is calculated relative to intraspecific competition intensity (Grace 1993; Turkington et al. 1993). For the invader in our study, *A. capillaris*, such universal superior performance over co-occurring native species suggests that *A. capillaris* may be a rare "super-invader" (Daehler 2003). This could partly explain its increasing dominance in native New Zealand grasslands since the 1960s (Rose 1995; Rose et al. 2004).

ABOVE VS. BELOWGROUND COMPETITION

The invasive grass's superior competitive ability, along with its negative impact on native plant species, was driven by better capture of belowground resources. Evidence for this comes from the decreases in total N content and increases in C: N ratios of native species in the belowground competition treatment relative to the control treatment, along with the co-occurring increases in total N content of the invader. This clearly demonstrates that belowground competition allowed the invader to capture key resources required for plant growth, in this case soil nitrogen, which the native species would otherwise have acquired. It is also possible that release from belowground intraspecific competition allowed the invader to grow faster and larger. In contrast, when native species were released from belowground intraspecific competition their biomass decreased (fig. 1). The only two other studies that separated above and belowground competition explicitly between native and invasive species also both reported that belowground competition was more important in mediating invader impacts than aboveground competition (Dillenburg et al. 1993; Kueffer et al. 2007). However, these experiments tested functionally dissimilar plant species, namely lianas and trees (Dillenburg et al. 1993) or adult trees and saplings (Kueffer et al. 2007), which means their results are influenced by differences in lifeform and stage (Vila and Weiner 2004). Our results extend the findings of these previous studies by showing that belowground competition is a key driver of invasive plant impacts within a single plant functional group, life form and growth stage. In the broader literature, belowground competition has been shown to play a crucial role in structuring plant communities (Fargione et al. 2003; Harpole and Tilman 2006; Hillerislambers et al. 2012) and is often more important in determining competitive outcomes than aboveground competition (Wilson 1988). Nevertheless, the two types of competition are likely to interact (Cahill 2002), and in addition to the direct impact of belowground competition in our study, there was also an indirect impact *via* the facilitation of aboveground competitive interactions. There are three lines of evidence for this. Firstly, the aboveground competition treatment had no effect on native or invasive species responses. Secondly, the full competition treatment had a stronger effect on native and invasive species total biomass responses than the belowground competition treatment. Thirdly, this greater impact on native species biomass responses was accompanied by an increase in invader aboveground biomass (Fig. 2b), suggesting the additional impact on native species is likely to have been driven by shading effects. Nonetheless, the impact of aboveground competition in the full competition treatment was still entirely reliant on the invader's superior capture of belowground resources.

The invasive grass's capacity to out-compete the native grasses for belowground resources may be related to its greater root biomass relative to the natives (Figs. 2c-d), since belowground competition appears to be size-symmetric (Cahill and Casper 2000). Root biomass, alongside other root traits, may be influential in driving invasive grass species success (Thomsen et al. 2006), which potentially makes it a useful screening tool. Nonetheless, root biomass alone is unlikely to determine belowground competitive outcomes between species (Cahill 2003), but typically interacts with other factors, such as feedbacks with soil biota (van der Putten et al. 2016). For example, belowground enemy release may also have facilitated the invasive grass's superior competitive abilities belowground (Agrawal et al. 2005; Reinhart and Callaway 2006). Likewise, allelopathic effects cannot be excluded; although we found no evidence in the literature that *A. capillaris* has allelopathic effects on co-occurring species. While the exact reason for *A. capillaris'* belowground superiority is difficult to determine, our results clearly demonstrate that belowground competition is central to delivering this invader's impact.

NITROGEN AVAILABILITY

While higher N-availability increased the aboveground biomass of the invasive grass but not the native species, it did not increase the invader's impact on native species. Nor did it diminish the relative importance of belowground competition. This contradicts our second hypothesis that *A. capillaris'* competitive impacts on native species, and the relative importance of aboveground competition over belowground competition, both increase with increasing N availability. In fact, native species showed significantly greater reductions in total N content, in response to belowground competition with the invader, under low N conditions than high N conditions. Furthermore, it was only under low N conditions that belowground competition with the invader altered native species tissue nutrient stoichiometry (i.e. increased their C: N ratios). Nitrogen was therefore likely a limiting resource in our experiment, as supported by various lines of evidence. Firstly, the exceptionally low N:P ratio (0.6) of the soil was lower than the critical N:P ratio in aboveground vegetation (ca. 15);

below which growth is limited by N (Olde Venterink et al. 2003; Olde Venterink and Güsewell 2010). Secondly, nitrogen addition, in the high N treatment, alleviated the negative impact of belowground competition on native species N capture rates but not their biomass. This suggests the invader out-competed the natives for other belowground resources in the high N treatment, which are essential for growth and either became limiting following N addition, or were co-limiting (Harpole et al. 2011). It is surprising that N addition did not enhance invader impact as non-native grass invasions in New Zealand, and elsewhere, are often associated with increased N availability (Dickie et al. 2014; Seabloom et al. 2015). Furthermore, native grasses that are adapted to low fertility soils, such as those in our study, may not respond to added nutrients, or in some cases may even respond negatively; unlike exotic invasive grasses which often respond positively (Thompson and Leishman 2004; Leishman and Thomson 2005; Radford et al. 2007; Seabloom et al. 2015). However, in its native range, *A. capillaris* is not considered a high-N species (Hill et al. 1999) and invasive plants can also succeed in low fertility environments (Funk and Vitousek 2007; Heberling and Fridley 2016), despite some efforts to restore native species by soil fertility reduction (e.g. Blumenthal et al. 2003). Invasive plants that out-compete co-occurring native species across wide gradients of soil fertility, such as *A. capillaris* in New Zealand grasslands, are likely to have the highest net impacts on native plant communities.

CONCLUSION

The invasive grass *A. capillaris* reduced co-occurring native grass species biomass by half, and their resource capture by up to 75%. These impacts were driven directly and indirectly by belowground, not aboveground, competition - regardless of N availability. The invader's greater root biomass appeared to facilitate its belowground competitive advantage. However, future studies could incorporate other root traits in order to improve our understanding of native – invasive plant root interactions, as this is currently underdeveloped in comparison with our knowledge of aboveground interactions. The overriding influence of belowground competition across wide levels of N availability in our study was unexpected, as the relative importance of aboveground competition often increases with increasing soil N availability (Wilson and Tilman 1991). Nevertheless, recent analyses reveal the prevalence of nutrient co-limitation across ecosystems globally, including grasslands (Harpole et al. 2011; Fay et al. 2015), which underlines the significance of belowground competition for multiple soil nutrients (Harpole and Tilman 2007; Harpole et al. 2016). In light of these findings, our results suggest that belowground competition may be under-appreciated as invasive plant impact mechanism, particularly in high fertility ecosystems, such as those experiencing N enrichment due to global change. Our findings could help to improve predictions of the impact on native species diversity of two pervasive, and interacting, drivers of global environmental change.

Acknowledgements

We would like to thank Karen Boot, Rowan Buxton and Chris Morse for field and logistical assistance, along with Matthew Clare and Silke Broadbent for help in the greenhouse, and Paul Chambre for experimental advice. AB is funded by a Lancaster University Faculty of Science and Technology studentship.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

Aerts R (1999) Interspecific competition in natural plant communities: mechanisms, trade-offs and plant–soil feedbacks. J Exp Bot 50:29–37. doi: 10.1093/jexbot/50.330.29

Agrawal A, Kotanen P, Mitchell C, et al (2005) Enemy release? An experiment with congeneric plant pairs and diverse above-and belowground enemies. Ecology 86:2979–2989.

Barney JN, Tekiela DR, Barrios-Garcia MN, et al (2015) Global Invader Impact Network (GIIN): Toward standardized evaluation of the ecological impacts of invasive plants. Ecol Evol 5:2878–2889. doi: 10.1002/ece3.1551

Barney JN, Tekiela DR, Dollete ESJ, Tomasek BJ (2013) What is the real impact of invasive plant species? Front Ecol Environ 11:322–329. doi: 10.1890/120120

Besaw LM, Thelen GC, Sutherland S, et al (2011) Disturbance, resource pulses and invasion: Short-term shifts in competitive effects, not growth responses, favour exotic annuals. J Appl Ecol 48:998–1006. doi: 10.1111/j.1365-2664.2011.01988.x

Blumenthal DM, Jordan NR, Russelle MP (2003) Soil carbon addition controls weeds and facilitates prairie restoration. Ecol Appl 13:605–615.

Bobbink R (1991) Effects of Nutrient Enrichment in Dutch Chalk Grassland. J Appl Ecol 28:28–41.

Borer ET, Seabloom EW, Gruner DS, et al (2014) Herbivores and nutrients control grassland plant diversity via light limitation. Nature 508:517–20. doi: 10.1038/nature13144

Broadbent AAD, Orwin KH, Peltzer DA, et al (2017) Invasive N-fixer Impacts on Litter Decomposition Driven by Changes to Soil Properties Not Litter Quality. Ecosystems 20:1–13. doi: 10.1007/s10021-016-0099-3

CABI (2017) Invasive Species Compendium. Wallingford, UK CAB Int. www.cabi.org/isc.

Cahill JF (2002) Interactions between root and shoot competition vary among species. Oikos

99:101–112. doi: 10.1034/j.1600-0706.2002.990111.x

Cahill JF (1999) Fertilization Effects on Interactions Between Above- and Belowground Competition. Ecology 80:466–480.

Cahill JF (2003) Lack of relationship between below-ground competition and allocation to roots in 10 grassland species. J Ecol 91:532–540.

Cahill JF, Casper BB (2000) Investigating the relationship between neighbor root biomass and belowground competition: field evidence for symmetric competition belowground. Oikos 90:311–320. doi: 10.1034/j.1600-0706.2000.900211.x

Craine JM, Lee WG (2003) Covariation in leaf and root traits for native and non-native grasses along an altitudinal gradient in New Zealand. Oecologia 134:471–8.

Daehler C (2003) Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. Annu Rev Ecol Evol Syst 34:183–211.

Daly GT (1964) Leaf-surface Wax in Poa colensoi. J Exp Bot 15:160–165.

Dickie IA, St John MG, Yeates GW, et al (2014) Belowground legacies of Pinus contorta invasion and removal result in multiple mechanisms of invasional meltdown. AoB Plants 6:1–15. doi: 10.1093/aobpla/plu056

Dillenburg LR, Whigham DF, Teramura AH, Forseth IN (1993) Effects of below- and aboveground competition from the vines Lonicera japonica and Parthenocissus quinquefolia on the growth of the tree host Liquidambar styraciflua.Oecologia 93:48–54.

DiTommaso A, Aarssen LW (1991) Effect of nutrient level on competition intensity in the field for three coexisting grass species. J Veg Sci 2:513–522. doi: 10.2307/3236033

Duncan RP, Webster RJ, Jensen CA (2001) Declining plant species richness in the tussock grasslands of Canterbury and Otago , South Island , New Zealand. New Zealand Journal Ecology 2:35–47.

Edgar E, Connor H (2000) Flora of New Zealand. Volume V. Manaaki Whenua Press

Edgar E, Forde MB (1991) Agrostis L. in New Zealand. New Zealand Journal Botany 29:139–161. doi: 10.1080/0028825X.1991.10416717

Fargione J, Brown CS, Tilman D (2003) Community assembly and invasion: An experimental test of neutral versus niche processes. PNAS 100:8916–8920.

Fay PA, Prober SM, Harpole WS, et al (2015) Grassland productivity limited by multiple nutrients. Nat Plants 1:15080. doi: 10.1038/nplants.2015.80

Firn J, Moore JL, MacDougall AS, et al (2011) Abundance of introduced species at home predicts abundance away in herbaceous communities. Ecol Lett 14:274–81

Funk JL, Vitousek PM (2007) Resource-use efficiency and plant invasion in low-resource systems. Nature 446:1079–81. doi: 10.1038/nature05719

Grace JB (1993) The effects of habitat productivity on competition intensity. Trends Ecol Evol 8:229–230. doi: 10.1016/0169-5347(93)90194-T

Harpole WS, Ngai JT, Cleland EE, et al (2011) Nutrient co-limitation of primary producer communities. Ecol Lett 14:852–862. doi: 10.1111/j.1461-0248.2011.01651.x

Harpole WS, Sullivan LL, Lind EM, et al (2016) Addition of multiple limiting resources reduces grassland diversity. Nature 537:1–9. doi: 10.1038/nature19324

Harpole WS, Tilman D (2006) Non-neutral patterns of species abundance in grassland communities. Ecol Lett 9:15–23. doi: 10.1111/j.1461-0248.2005.00836.x

Harpole WS, Tilman D (2007) Grassland species loss resulting from reduced niche dimension. Nature 446:791–3. doi: 10.1038/nature05684

Hautier Y, Niklaus P a, Hector A (2009) Competition for light causes plant biodiversity loss after eutrophication. Science 324:636–8. doi: 10.1126/science.1169640

Heberling JM, Fridley JD (2016) Invaders do not require high resource levels to maintain physiological advantages in a temperate deciduous forest. Ecology 97:874–884. doi: 10.1890/15-1659.1

Hill MO, Mountford JO, Roy DB, Bunce RGH (1999) Ellenberg's indicator values for British plants. ECOFACT Volume 2 Technical Annex.

Hillerislambers J, Adler PB, Harpole WS, et al (2012) Rethinking Community Assembly through the Lens of Coexistence Theory. Annu Rev Ecol Evol Syst 43:227–48.

Johnson NC, Rowland DL, Corkidi L, Allen EB (2008) PLANT WINNERS AND LOSERS DURING GRASSLAND N-EUTROPHICATION DIFFER IN BIOMASS ALLOCATION AND MYCORRHIZAS. Ecology 89:2868–2878.

Kueffer C, Schumacher E, Fleischmann K, et al (2007) Strong below-ground competition shapes tree regeneration in invasive Cinnamomum verum forests. J Ecol 95:273–282.

Lai HR, Mayfield MM, Gay-des-combes JM, et al (2015) Distinct invasion strategies operating within a natural annual plant system. Ecol Lett 18:336–346.

Leishman MR, Haslehurst T, Ares A, Baruch Z (2007) Leaf trait relationships of native and invasive plants: Community- and global-scale comparisons. New Phytol 176:635–643.

Leishman MR, Thomson VP (2005) Experimental evidence for the effects of additional water, nutrients and physical disturbance on invasive plants in low fertility Hawkesbury

Sandstone soils, Sydney, Australia. J Ecol 93:38–49.

Leishman MR, Thomson VP, Cooke J (2010) Native and exotic invasive plants have fundamentally similar carbon capture strategies. J Ecol 98:28–42.

Levine JM, Vilà M, D'Antonio CM, et al (2003) Mechanisms underlying the impacts of exotic plant invasions. Proc R Soc B Biol Sci 270:775–81. doi: 10.1098/rspb.2003.2327

MacDougall AS, Gilbert B, Levine JM (2009) Plant invasions and the niche. J Ecol 97:609–615. doi: 10.1111/j.1365-2745.2009.01514.x

Mark AF, McLennan B (2005) The conservation status of New Zealand's indigenous grasslands. New Zealand Journal Botany 43:245–270.

Olde Venterink H, Güsewell S (2010) Competitive interactions between two meadow grasses under nitrogen and phosphorus limitation. Funct Ecol 24:877–886.

Olde Venterink H, Wassen MJ, Verkroost AWM, De Ruiter PC (2003) Species richness-productivity patterns differ between N-, P-, and K-limited wetlands. Ecology 84:2191–2199. doi: 10.1890/01-0639

Ordonez A, Olff H (2013) Do alien plant species profit more from high resource supply than natives? A trait-based analysis. Glob Ecol Biogeogr 22:648–658.

Ordonez A, Wright IJ, Olff H (2010) Functional differences between native and alien species: a global-scale comparison. Funct Ecol 24:1353–1361.

R Core Team (2016) R: A language and environment for statistical computing.

Radford IJ, Dickinson KJM, Lord JM (2007) Functional and performance comparisons of invasive Hieracium lepidulum and co-occurring species in New Zealand. Austral Ecol 32:338–354. doi: 10.1111/j.1442-9993.2007.01700.x

Reinhart K, Callaway R (2006) Soil biota and invasive plants. New Phytol 445–457.

Rose A (1995) Vegetation change over 25 years in a New Zealand short-tussock grassland: effects of sheep grazing and exotic invasions. New Zealand Journal of Ecology 19(2): 163-174

Rose AB, Frampton CM (2007) Rapid short-tussock grassland decline with and without grazing, Marlborough, New Zealand. New Zealand Journal of Ecology 31:232–244.

Rose AB, Suisted PA, Frampton CM (2004) Recovery, invasion, and decline over 37 years in a Marlborough short tussock grassland, New Zealand. New Zealand Journal of Botany 42:77–87. doi: 10.1080/0028825X.2004.9512891

Scott D (2000) Fertiliser and grazing rejuvenation of fescue tussock grassland. New Zealand Journal Agricultural Research 43:481–490. doi: 10.1080/00288233.2000.9513444

Seabloom EW, Borer ET, Buckley YM, et al (2015) Plant species' origin predicts dominance and response to nutrient enrichment and herbivores in global grasslands. Nat Commun 6:7710. doi: 10.1038/ncomms8710

Seabloom EW, Harpole WS, Reichman OJ, Tilman D (2003) Invasion, competitive dominance, and resource use by exotic and native California grassland species. Proc Natl Acad Sci U S A 100:13384–9. doi: 10.1073/pnas.1835728100

Stevens CJ, Dise NB, Mountford JO, Gowing DJ (2004) Impact of nitrogen deposition on the species richness of grasslands. Science 303:1876–9. doi: 10.1126/science.1094678

Thompson VP, Leishman MR (2004) Survival of native plants of Hawkesbury Sandstone communities with additional nutrients: effect of plant age and habitat type. Aust J Bot 52:141–147.

Thomsen MA, Corbin JD, D'Antonio CM (2006) The effect of soil nitrogen on competition between native and exotic perennial grasses from northern coastal California. Plant Ecol 186:23–35. doi: 10.1007/s11258-006-9109-4

Tilman D (1988) Plant strategies and the dynamics and structure of plant communities. Princeton University Press, Princeton, New Jersey, USA.

Tilman D (1982) Resource Competition and Community Structure. Princeton University Press. Princeton, USA.

Trinder CJ, Brooker RW, Robinson D (2013) Plant ecology's guilty little secret: Understanding the dynamics of plant competition. Funct Ecol 27:918–929.

Turkington R, Klein E, Chanway CP (1993) Interactive effects of nutrients and disturbance: an experimental test of plant strategy theory. Ecology 74:863–878.

Tylianakis JM, Didham RK, Bascompte J, Wardle D a. (2008) Global change and species interactions in terrestrial ecosystems. Ecol Lett 11:1351–1363.

van der Putten WH, Bradford MA, Pernilla Brinkman E, et al (2016) Where, when and how plant-soil feedback matters in a changing world. Funct Ecol 1109–1121.

Van Kleunen M, Dawson W, Essl F, et al (2015) Global exchange and accumulation of non-native plants. Nature 525:100–103. doi: 10.1038/nature14910

van Kleunen M, Weber E, Fischer M (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. Ecol Lett 13:235–45.

Vilà M, Espinar JL, Hejda M, et al (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecol Lett 14:702–8. doi: 10.1111/j.1461-0248.2011.01628.x

Vila M, Weiner J (2004) Are invasive plant species better competitors than native plant species?–evidence from pair-wise experiments. Oikos 105: 229-238

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997) Human Domination of Earth's Ecosystems. Science 277:494–499. doi: 10.1126/science.277.5325.494

Williams PA (1998) Response of broom (Cytisus scoparius) to control measures. Sci Conserv report, Dep Conserv New Zeal 97.

Wilsey BJ, Polley WH (2006) Aboveground productivity and root-shoot allocation differ between native and introduced grass species. Oecologia 150:300–309.

Wilson JB (1988) SHOOT COMPETITION AND ROOT COMPETITION. J Appl Ecol 25:279–296.

Wilson SD, Shay JM (1990) Competition, Fire, and Nutients in a Mixed-Grass Prairie. Ecology 71:1959–1967. doi: 10.2307/1937604

Wilson SD, Tilman D (1991) Component of Plant Competition Along an Experimental Gradient of Nitrogen Availability. Ecology 72:1050–1065. doi: 10.2307/1940605

Tables

Table 1 Results of 3-way ANOVAs testing effects of native species identity (NSI), competition (C), nitrogen addition (N+) and their interactions on biomass responses (total, aboveground and belowground) of native (*Poa cita* and *Poa colensoi*) and invasive species (*Agrostis capillaris*). All factors are fixed effects. Biomass data were log-transformed before analysis.

		Total biomass		Aboveground biomass		Belowground biomass	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Native species							
NSI	1	222.5	<0.01	221.1	<0.01	178.2	<0.01
C	3	24.6	<0.01	18.7	<0.01	30.8	<0.01
N+	1	0.5	0.48	0.4	0.54	10.8	<0.01
NSI * C	3	0.8	0.51	0.9	0.47	4.3	<0.01
NSI * N+	1	1.0	0.31	1.2	0.28	1.6	0.21
C * N+	3	0.3	0.80	0.6	0.61	0.6	0.60
NSI * C * N+	3	0.8	0.51	0.7	0.57	1.5	0.21
Invasive species							
NSI	1	2.1	0.15	4.9	0.03	0.03	0.86
C	3	66.0	<0.01	49.1	<0.01	23.0	<0.01
N+	1	1.9	0.18	30.1	<0.01	26.3	<0.01
NSI * C	3	1.4	0.25	1.3	0.28	0.7	0.53
NSI * N+	1	0.7	0.42	0.1	0.82	0.8	0.37
C * N+	3	1.3	0.28	2.3	0.09	0.2	0.93
NSI * C * N+	3	1.3	0.29	0.4	0.77	1.5	0.22

Table 2 Results of 3-way ANOVAs testing effects of native species identity (NSI), competition (C), nitrogen addition (N+) and their interactions on % dead aboveground (AG) biomass, total N content and C/N ratio of native (*P. cita* and *P. colensoi*) and invasive species (*A. capillaris*). All factors are fixed effects. Total N content and CN ratio data were log-transformed before analysis.

		% Dead AG biomass		Total N content		C/N ratio	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Native species							
NSI	1	1.7	0.20	154.0	<0.01	0.2	0.68
C	3	44.7	<0.01	56.6	<0.01	4.5	<0.01
N+	1	0.1	0.74	65.9	<0.01	206.2	<0.01
NSI * C	3	0.2	0.87	2.8	0.05	1.7	0.18
NSI * N+	1	0.4	0.54	13.5	<0.01	28.7	<0.01
C * N+	3	1.4	0.25	3.5	0.02	14.8	<0.01
NSI * C * N+	3	0.7	0.57	1.1	0.36	3.3	0.03
Invasive species							
NSI	1	0.2	0.63	1.7	0.20	<0.1	0.87
C	3	14.4	<0.01	79.8	<0.01	4.0	0.01
N+	1	0.6	0.43	668.2	<0.01	1233.0	<0.01
NSI * C	3	1.1	0.35	0.7	0.53	0.9	0.42
NSI * N+	1	2.3	0.13	0.03	0.86	0.5	0.49
C * N+	3	0.3	0.82	1.1	0.36	1.3	0.27
NSI * C * N+	3	2.6	0.06	1.1	0.35	1.3	0.29

Table 3 Results of 2-way ANOVAs testing effects of competition (C), nitrogen addition (N+) and their interaction on CN ratio of native species (*P. cita* and *P. colensoi*). All factors are fixed effects. *P. cita* data were log-transformed before analysis.

	CN ratio		
	df	*F*	*P*
P. cita			
C	3	4.1	0.01
N+	1	218.2	<0.01
C * N+	3	3.1	0.04
P. colensoi			
C	3	4.1	0.01
N+	1	37.0	<0.01
C * N+	3	13.7	<0.01

Table 4 Results of 3-way ANOVAs testing effects of native species identity (NSI), competition (C), nitrogen addition (N+) and their interactions on root mass fraction (RMF) and root nitrogen fraction (RNF) of native (*P. cita* and *P. colensoi*) and invasive species (*A. capillaris*). All factors are fixed effects. Invasive species data were log-transformed before analysis.

		RMF		RNF	
	df	*F*	*P*	*F*	*P*
Native species					
NSI	1	<0.1	0.82	5.9	0.02
C	3	4.7	<0.01	3.2	0.03
N+	1	20.5	<0.01	22.7	<0.01
NSI * C	3	0.3	0.84	2.4	0.07
NSI * N+	1	0.1	0.82	0.3	0.58
C * N+	3	0.7	0.57	0.4	0.77
NSI * C * N+	3	1.8	0.16	0.9	0.44
Invasive species					
NSI	1	2.4	0.12	0.2	0.69
C	3	0.7	0.53	1.9	0.14
N+	1	69.2	<0.01	74.6	<0.01
NSI * C	3	0.7	0.56	0.2	0.87
NSI * N+	1	0.3	0.61	0.5	0.50
C * N+	3	1.3	0.30	0.7	0.56
NSI * C * N+	3	0.5	0.66	1.3	0.28

Figure legends

Fig. 1 Schematic diagram showing experimental setup for invasive (I) and native (N) species planted in pots with above and belowground partitions to create four competition treatments: a) Control - full intraspecific competition between a pair of invasive and a pair native individuals, but no interspecific competition; b) Aboveground - aboveground interspecific competition between invasive and native individuals, and belowground intraspecific competition; c) Belowground - belowground interspecific competition between invasive and native individuals and aboveground intraspecific competition and d) Full - above and belowground interspecific competition between invasive and native individuals but no intraspecific competition. In a) and d) the above and belowground partitions were flush; they are depicted lightly apart for clarity.

Fig. 2 Biomass responses of native and invasive species to four competition treatments (See Fig. 1 legend for full description). a) Total biomass; b) aboveground biomass for native and invasive species; c) below-ground biomass for invasive species; d) below-ground responses of two native species, *P. cita* and *P. colensoi* to these treatments. Box-and-whisker plots show individual data points, means, one standard error and range of data. Means with the same letter are not significantly different ($p > 0.05$, Tukey HSD post hoc tests); in a) and b) upper case letters are for ANOVAs comparing invader responses; lower case for native species responses

Fig. 3 Resource capture and allocation responses of native and invasive species to four competition treatments (See Fig. 1 legend for full description). a) Total N content for invasive species; b) total N content for native species in high or low N treatments; c) C/N ratio for *P. cita* in different N treatments; d) C/N ratio for *P. colensoi* in different N treatments; e) C/N ratio for invasive species; f) root mass fraction (RMF) for native and invasive species. Box-and-whisker plots show individual data points, means, one standard error and range of data. Means with the same letter are not significantly different ($p > 0.05$, Tukey HSD post hoc tests); in f) upper case letters are for ANOVAs comparing invader responses; lower case for native species responses

Fig. 1

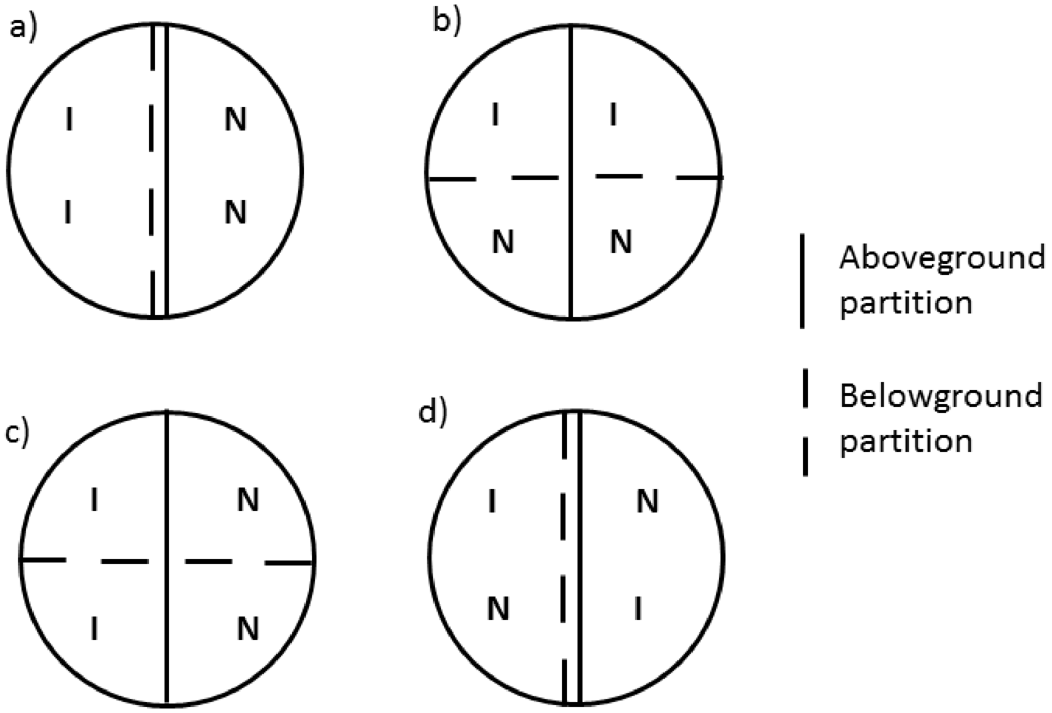

a)
I
N
I
N
b)
I
I
N
N
c)
I
N
I
N
d)
I
N
N
I
Aboveground partition
Belowground partition

Fig. 2

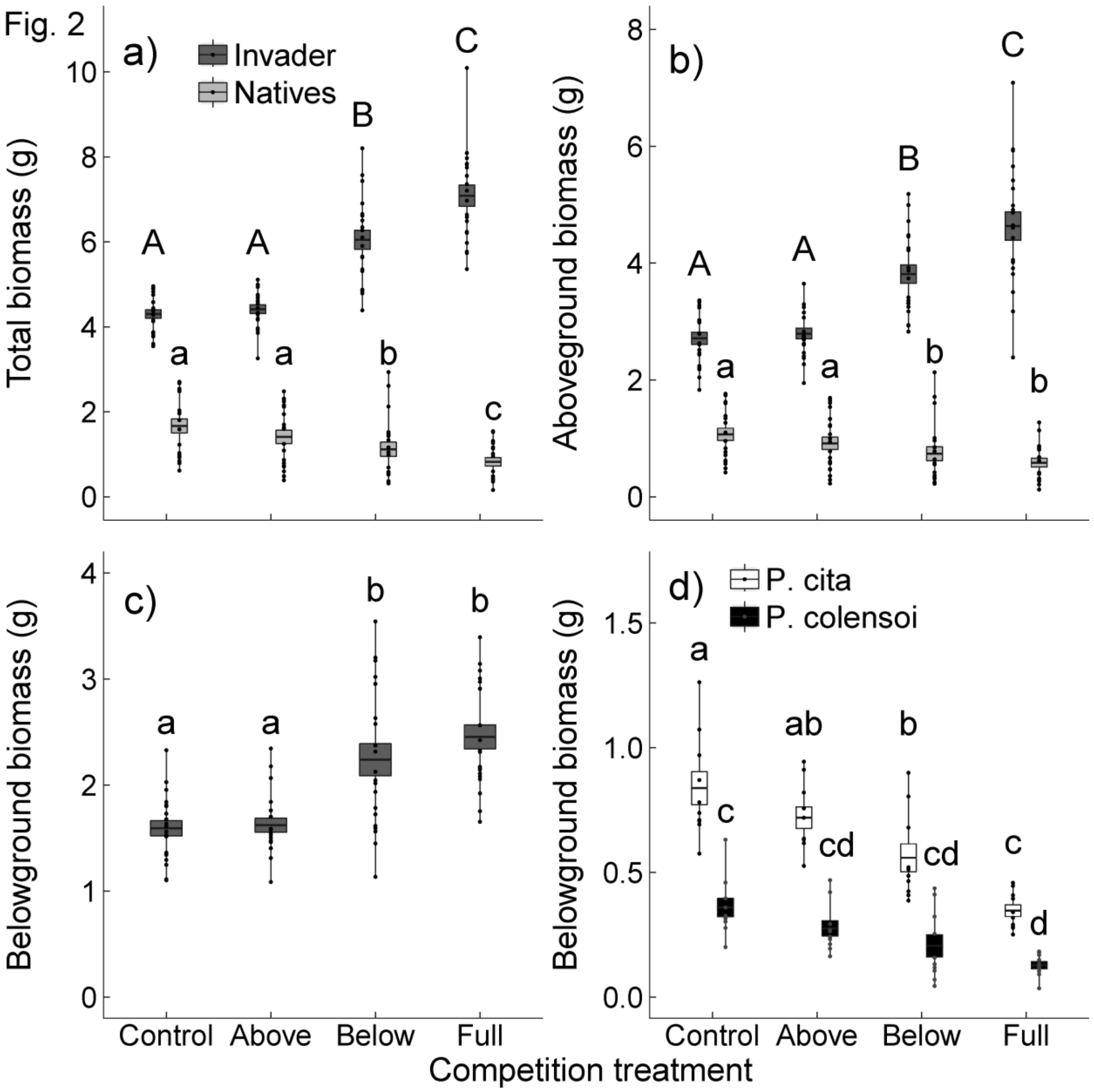
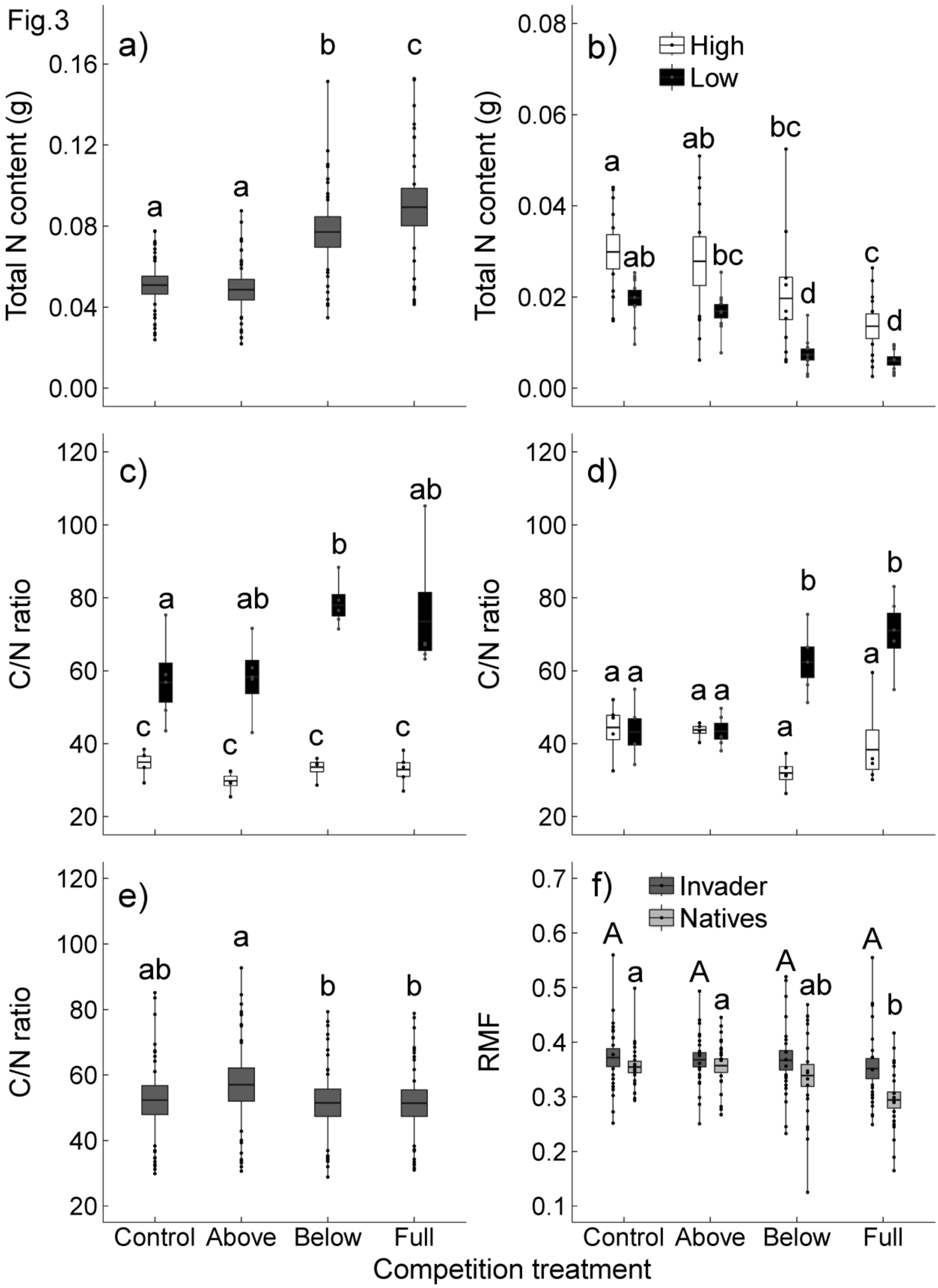

a)
Invader
Natives
Total biomass (g)
0
2
4
6
8
10
A
A
B
C
a
a
b
c
b)
Aboveground biomass (g)
0
2
4
6
8
A
A
B
C
a
a
b
b
c)
Belowground biomass (g)
0
1
2
3
4
a
a
b
b
d)
P. cita
P. colensoi
Belowground biomass (g)
0.0
0.5
1.0
1.5
a
ab
b
c
c
cd
cd
d
Control
Above
Below
Full
Control
Above
Below
Full
Competition treatment

Fig.3


a)
Total N content (g)
0.00
0.04
0.08
0.12
0.16
a
a
b
c
b)
High
Low
Total N content (g)
0.00
0.02
0.04
0.06
0.08
a
ab
ab
bc
bc
d
c
d
c)
C/N ratio
20
40
60
80
100
120
c
a
c
ab
c
b
c
ab
d)
C/N ratio
20
40
60
80
100
120
a
a
a
a
a
b
a
b
e)
C/N ratio
20
40
60
80
100
120
ab
a
b
b
f)
Invader
Natives
RMF
0.1
0.2
0.3
0.4
0.5
0.6
0.7
A
a
A
a
A
ab
A
b
Control
Above
Below
Full
Control
Above
Below
Full
Competition treatment