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

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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.
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 C₃ tussock grasses native to New Zealand: *P. cita* and *P. colensoi*. *A. capillaris* is a Eurasian rhizomatous perennial C₃ 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 NH₄NO₃ 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 Cytisus 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 (NO₃⁻-N and NH₄⁺-N) was 2.97 ± 0.15 µg N g soil⁻¹ and mean
NaCO₃ extractable PO₄⁻-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 °C: 16 °C. 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 °C 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 °C 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 <
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*’ 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.

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**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.

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<tr>
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<td>3</td>
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<td>&lt;0.01</td>
<td>18.7</td>
<td>&lt;0.01</td>
<td>30.8</td>
</tr>
<tr>
<td>N+</td>
<td>1</td>
<td>0.5</td>
<td>0.48</td>
<td>0.4</td>
<td>0.54</td>
<td>10.8</td>
</tr>
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<td>3</td>
<td>0.8</td>
<td>0.51</td>
<td>0.9</td>
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</tr>
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<td>1</td>
<td>1.0</td>
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</tr>
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<td>0.80</td>
<td>0.6</td>
<td>0.61</td>
<td>0.6</td>
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<td>0.8</td>
<td>0.51</td>
<td>0.7</td>
<td>0.57</td>
<td>1.5</td>
</tr>
<tr>
<td>Invasive species</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSI</td>
<td>1</td>
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<td>4.9</td>
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<td>0.03</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
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<td>49.1</td>
<td>&lt;0.01</td>
<td>23.0</td>
</tr>
<tr>
<td>N+</td>
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<td>1.9</td>
<td>0.18</td>
<td>30.1</td>
<td>&lt;0.01</td>
<td>26.3</td>
</tr>
<tr>
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<td>3</td>
<td>1.4</td>
<td>0.25</td>
<td>1.3</td>
<td>0.28</td>
<td>0.7</td>
</tr>
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<td>0.1</td>
<td>0.82</td>
<td>0.8</td>
</tr>
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<td>0.28</td>
<td>2.3</td>
<td>0.09</td>
<td>0.2</td>
</tr>
<tr>
<td>NSI * C * N+</td>
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<td>1.3</td>
<td>0.29</td>
<td>0.4</td>
<td>0.77</td>
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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.

<table>
<thead>
<tr>
<th></th>
<th>% Dead AG biomass</th>
<th>Total N content</th>
<th>C/N ratio</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Native species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1.7</td>
<td>0.20</td>
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<tr>
<td>C</td>
<td>3</td>
<td>44.7</td>
<td>&lt;0.01</td>
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<tr>
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<td>0.74</td>
</tr>
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<td>0.2</td>
<td>0.87</td>
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<tr>
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<td>1</td>
<td>0.4</td>
<td>0.54</td>
</tr>
<tr>
<td>C * N+</td>
<td>3</td>
<td>1.4</td>
<td>0.25</td>
</tr>
<tr>
<td>NSI * C * N+</td>
<td>3</td>
<td>0.7</td>
<td>0.57</td>
</tr>
<tr>
<td>Invasive species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSI</td>
<td>1</td>
<td>0.2</td>
<td>0.63</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
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<td>&lt;0.01</td>
</tr>
<tr>
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<td>0.43</td>
</tr>
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<td>1.1</td>
<td>0.35</td>
</tr>
<tr>
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<td>2.3</td>
<td>0.13</td>
</tr>
<tr>
<td>C * N+</td>
<td>3</td>
<td>0.3</td>
<td>0.82</td>
</tr>
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<td>NSI * C * N+</td>
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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.

<table>
<thead>
<tr>
<th>CN ratio</th>
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<th>P</th>
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</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td><em>P. cita</em></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>4.1</td>
<td>0.01</td>
</tr>
<tr>
<td>N+</td>
<td>1</td>
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</tr>
<tr>
<td>C * N+</td>
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<td>3.1</td>
<td>0.04</td>
</tr>
<tr>
<td><em>P. colensoi</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>4.1</td>
<td>0.01</td>
</tr>
<tr>
<td>N+</td>
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</tr>
<tr>
<td>C * N+</td>
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<td>13.7</td>
<td>&lt;0.01</td>
</tr>
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</table>
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.

<table>
<thead>
<tr>
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<th>RMF</th>
<th></th>
<th>RNF</th>
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</thead>
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<td></td>
<td>df</td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td><strong>Native species</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSI</td>
<td>1</td>
<td>&lt;0.1</td>
<td>0.82</td>
<td>5.9</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>4.7</td>
<td>&lt;0.01</td>
<td>3.2</td>
</tr>
<tr>
<td>N+</td>
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<td>&lt;0.01</td>
<td>22.7</td>
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<tr>
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</tr>
<tr>
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<td>0.1</td>
<td>0.82</td>
<td>0.3</td>
</tr>
<tr>
<td>C * N+</td>
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<td>0.7</td>
<td>0.57</td>
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<tr>
<td>NSI * C * N+</td>
<td>3</td>
<td>1.8</td>
<td>0.16</td>
<td>0.9</td>
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<tr>
<td><strong>Invasive species</strong></td>
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</tr>
<tr>
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<td>0.12</td>
<td>0.2</td>
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<tr>
<td>C</td>
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<td>0.53</td>
<td>1.9</td>
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<tr>
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<td>&lt;0.01</td>
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<td>0.3</td>
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<td>C * N+</td>
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<td>1.3</td>
<td>0.30</td>
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<td>NSI * C * N+</td>
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<td>0.5</td>
<td>0.66</td>
<td>1.3</td>
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</tbody>
</table>
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)**
  
  ![Diagram a)

- **b)**
  
  ![Diagram b)

- **c)**
  
  ![Diagram c)

- **d)**
  
  ![Diagram d)

Aboveground partition

Belowground partition