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# Resistance of plant communities to invasion by tall fescue: An experimental study combining species diversity, functional traits and nutrient levels



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# Abstract

The grass *Festuca arundinacea* is often planted for slope stabilisation in South Korea, and is spreading widely beyond the introduction sites. This study used a functional group approach to examine the resistance of plant combinations to invasion by *F. arundinacea* based on the limiting similarity and diversity-resistance hypotheses, and to elucidate the process of colonisation. The study simulated the environment of construction sites and surrounding areas that might be encountered by expanding populations of *F. arundinacea*. The role of nutrient condition in the ranking of functional group competitive ability was also examined. Twelve native plant species were categorised into three functional groups using combinations of functional traits. Pairwise (one-to-one competition), multiple (four different neighbouring species) and monoculture experimental settings were designed using two nutrient levels. The Relative Competition Index was used to interpret the competitive effect of neighbouring species on *F. arundinacea*. Species of the same functional group as *F. arundinacea* were unable to resist invasion, but annual plants with niche preemption ability could outcompete it. Competitive relationships between native plants and *F. arundinacea* were explained partially by functional group identity but were inconsistent with the limiting similarity hypothesis and the diversity-interaction. Unforeseen interactions within the artificial communities also produced unexpected effects. In designing artificial plant communities, it is necessary to consider functional traits that reflect the species characteristic of particular periods and indirect effects that modify the interaction between other species.

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# Introduction

There is a growing demand for restoration of damaged ecosystems, but factors inherent to the restoration process have the potential to cause further disturbance to the ecosystem. One possibility is the proliferation of invasive species in disturbed areas. Amongst plants, invasive alien species are a major threat to global biodiversity, causing problems by inhibiting the growth of native species and by alteration of community structure, thereby inducing changes in ecosystem functioning (Dassonville et al., 2008; Ehrenfeld, 2010; MEA, 2005; Pyšek et al., 2012; Vilà et al., 2011; Vitousek, 1994).

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In the regeneration of road-cut slopes, mixtures of plant seeds are sown by the spraying method. Pioneer plant species introduced for slope regeneration are intended to stabilise the ground quickly and provide a basis for the establishment of later successional species. However, if the introduced species are competitively dominant, they may prove difficult to control, inhibiting natural succession and so posing a major challenge to restoration. Around the world, ornamental plants and alien species introduced for cut-slope stabilisation commonly proliferate and disturb natural ecosystems (Dehnen-Schmutz, 2011; Park, Kim, Byun, Hong, & Lee, 2021).

Festuca arundinacea Schreber is a grass commonly known as "tall fescue", which has been introduced and utilised for slope stabilisation in South Korea. It forms a rhizome that can bind the soil and stabilise the ground (USDA, 2006). Its tenacious growth form makes it an excellent fit for slope greening, but also confers a strong potential to spread out from the slopes, forming monospecific landscapes. In South Korea, robust grasses such as F. arundinacea (tall fescue), Poa pratensis (Kentucky bluegrass), Eragrostis curvula (weeping lovegrass) and Dactylis glomerata (orchardgrass) have persisted and obstructed succession for decades after slope regeneration (Kil & Kim, 2014; Song et al., 2005). Festuca arundinacea has formed persistent monospecific landscapes and has spread widely beyond the introduction sites (Chung et al., 2015; Jung et al., 2017). However, the colonisation process and interactions with neighbouring species have not yet been studied.

Restoration ecology aims at desirable and reliable outcomes in terms of securing high biodiversity and stable ecosystem function, and it is therefore necessary to obtain predictable results when restoring damaged ecosystems (Benavas, Newton, Diaz, & Bullock, 2009; SER, 2002; Zirbel, Bassett, Grman, & Brudvig, 2017). In this context, plant functional traits may provide clues to understanding the mechanisms of community reassembly and ecosystem functioning during restoration (Byun, de Blois, & Brisson, 2013; Funk, Cleland, Suding, & Zavaleta, 2008). Functional traits reflect the mechanisms by which organisms use resources, which are directly or indirectly involved in species growth, reproduction and establishment (Cadotte, Carscadden, & Mirotchnick, 2011; Mouillot, Graham, Villéger, Mason, & Bellwood, 2013; Violle et al., 2007). Therefore, functional traits can be useful tools for interpreting, predicting and linking the assembly and functioning of communities and ecosystems (Cadotte, Arnillas, Livingstone, & Yasui, 2015; Lavorel et al., 2002; Naeem, Duffy, & Zavaleta, 2012; Zirbel et al., 2017).

Recently, attempts have been made to use functional diversity concepts to build communities resistant to invasive species (Byun, de Blois, & Brisson, 2015; Laughlin, 2014; Laughlin et al., 2018; Yannelli, Karrer, Hall, Kollmann, & Heger, 2018). The achievement of biotic resistance based on functional traits rests on application of the limiting similarity hypothesis and diversity-resistance hypothesis (Byun et al.,

2013; Funk et al., 2008). Specifically, the limiting similarity hypothesis proposes that intense competition may occur when two or more species use a similar strategy to exploit resources (Hardin, 1960; Macarthur & Levins, 1967). The diversity-resistance hypothesis proposes that a plant community containing more species has few or no empty niches, and is therefore more resistant to invasion (Elton, 1958). Since functional traits are related to the way nutrients are exploited (Cornelissen et al., 2003; Reich, 2014; Violle et al., 2007), the species in a functional group that have a similar role in a community would tend to compete with each other, depleting the resources used in common (Funk et al., 2008). When a community is viewed in functional group terms, biotic resistance can be enhanced by species combinations which include a larger number of functional group identities.

Resource competition is one of the main driving forces of plant community assembly (Grime, 1973; Newman, 1973). Resource utilisation strategies are a major factor influencing the establishment of species (Elton, 1958; Tilman, 1997). Differences in nutrient uptake capacity between invasive and native species contribute to differential growth rates (Funk, 2013; Gioria & Osborne, 2014). Settlement patterns of plant species can also vary depending on soil nutrient levels (Vilà & Weiner, 2004; White, Wilson, & Clarke, 2006). Although the functional group concept is closely related to resource exploitation patterns, studies examining the interplay of functional group identity and nutrients have not yet been reported.

This study examined the colonisation process of F. arundinacea and biotic interactions between F. arundinacea and the native neighbouring plants during the early stages of establishment. Our basic hypothesis was that biotic effects of neighbouring plants on F. arundinacea would be predictable from their functional traits. We tested the invasionresistance of plant combinations against F. arundinacea with respect to functional group identity and the predictions of the diversity-resistance hypothesis. Experimental conditions were designed to simulate the nutrient-poor cut slopes on which F. arundinacea is frequently established in Korea, and the nutrient-rich environments into which it may spread. We hypothesised that (1) multi-species combinations including diverse functional traits more effectively exploit common resources and inhibit the growth of the focal species by functional redundancy, and (2) nutrient levels strengthen or reverse the interspecies relationships within the invasionresistance design.

# Materials and methods

#### Plant selection and functional classification

Twenty-three plant species, which commonly coexist with *F. arundinacea* at major dispersal sources such as revegetated roadcut slopes, were selected to represent the

Trait	FG1	FG2	FG3	Source (Korea National Arboretum, 2017; Lee (2014))		
Disseminule form	D4, D3	D1, D4	D1, D2, D3, D4			
Dormancy form	M, N, MM	Th	Ch, H, G, Th Raunkiær (1934)			
Growth form	e, b	ps, pr, e, b	pr, e, t, l, b Raunkiær (1934)			
Radicoid form	R5	R5	R2, R3, R5	Numata (1970)		
Cotyledon	Dicotyledon	Dicotyledon	Monocotyledon, Dicotyledon	(Korea National Arboretum, 2017; Lee (2014))		
Duration	Perennial	Annual	Perennial	(Korea National Arboretum, 2017; Lee (2014))		
Seed mass (g*)	$13.65\pm12.82$	$0.50\pm0.94$	$1.67 \pm 1.49$	(Korea National Arboretum, 2017; Lee (2014))		
*thousand grain weight						
Height at maturity (m)	$7.35\pm9.90$	$0.86\pm0.40$	$0.94 \pm 0.68$	(Korea National Arboretum, 2017; Lee (2014))		
Woodiness	Woody	Non-woody	Non-woody, Woody (Korea National Arboretum, 2017; Lee (2014))			

Table 1. Summary of traits used to define the three functional groups.

D1: disseminated widely by wind or water; D2: disseminated by attachment to, or eaten by, animals or humans; D3: disseminated by mechanical propulsion of fruit dehiscence; D4: no special modification for dissemination.

R1: widest extent of rhizomatous growth; R2: moderate extent of rhizomatous growth; R3: narrowest extent of rhizomatous growth; R4: clonal growth by stolons and struck roots; R5: non-clonal growth.

MM: Megaphanerophyte; M: Microphanerophyte; N: Nanophanerophyte; Ch: Chamaephyte; H: Hemicryptophyte; G: Geophyte; HH: Hydrophyte; Th: Therophyte.

e: erect form; b: branched form; t: tussock form; l: liane form; p: procumbent form; r: rosette form; pr: partial-rosette form; ps: pseudo-rosette form.

functional spectrum of plant traits (Park et al., 2021; Song et al., 2005). These species were classified into three functional groups using nine traits related to plant establishment and competitive ability (Byun et al., 2017; Funk et al., 2008; Mwangi et al., 2007; Yannelli et al., 2018). Data on seed mass, height at maturity, woodiness, longevity, dispersal mode, and cotyledon form were compiled from the Korean Plant Names Index (Korea National Arboretum, 2017) and Lee (2014). Dormancy and growth forms were derived from Raunkiær's life form classification (Raunkiær, 1934) and radicoid form from Numata (1970). Continuous variable traits were represented by the median value of a gauged trait for each species to form a speciestrait matrix. Functional traits were investigated using Gower's similarity coefficient (Gower, 1971; Podani, 1999).

Plant species were sorted by cluster analysis with the ward option using the hclust function in the R statistical package. Species in functional group (FG) 1 were perennial woody plants, species in FG 2 were annual forbs, and those in FG 3 were perennial plants (Table 1 and Appendix A). After that, seeds of 23 species were subjected to a pre-germination test. Seeds used in the experiment were purchased from local seed suppliers except for those of Metaplexis japonica, which were collected from the roadside in Gyeonggi-do province in January 2018. All seeds were stored in the dark at 4 °C before the germination test according to the method of Lindig-Cisneros and Zedler (2001). Fifty seeds were placed in each of two Petri dishes, with Whatman filter paper No. 1 moistened with 5 ml of distilled water, under fluorescent light. Species for which germination rate was less than 5% were excluded. Finally, twelve neighbour plant species were selected, including Cirsium setidens, a Korean endemic species. Small-seeded plants were further sorted according to seed mass (thousand-grain weight, < 0.13 g) when seed mass was judged to play a key role in the experiment (Appendix B). Species nomenclature and status (native or introduced) followed that of Lee (2014).

#### **Experimental design**

The effects of neighbour plants on *F. arundinacea* were studied by the additive method in a mesocosm experiment (Byun et al., 2013; Connolly, Wayne, & Bazzaz, 2001; Snaydon, 1991). All combinations were replicated three times in a two-way factorial design with soil fertility and functional group combination.

Pairwise (F. arundinacea and one neighbour species), multispecies (F. arundinacea and four different neighbour species from each functional group) and monoculture (F. arundinacea only) pots were established (Appendix C). Pairwise pots were set up to test the interactive effects of the neighbour species and the focal species by using functional group identity and species identity. In the pairwise experiments, seeds of the native and focal species were sown at a 1:1 ratio. Multi-species pots were set up to simulate the situation in which seeds of F. arundinacea and the other species were sown on cut slopes, or where F. arundinacea had spread into the surrounding ecosystem at an early stage of establishment. In the multi-species settings, the functional trait-based combinations of four native species were designed to test whether they would inhibit the growth of F. arundinacea. Four native species in total randomly selected from three functional groups were sown with the focal species at the same rate. Finally, multi-species experiments were conducted using eight different combinations of randomly selected species (Appendix D).

All pots were sown with 5 g seeds, divided in proportion to the number of species. For example, there were 2.5 g seeds of neighbour species and 2.5 g seeds of F.

arundinacea in a pairwise competition. In a multi-species pot, each 1 g seeds of species A, B, C and D was sown with 1 g seeds of F. arundinacea. Since seed mass varies amongst species, this could potentially lead to differences in density of each species. However, the trade-off between seed mass and the number of seeds produced per plant (Jakobsson & Eriksson, 2000; Smith & Fretwell, 1974) is an intrinsic part of the reproductive and survival strategies of plant species (Henery & Westoby, 2001; Moles & Westoby, 2006). Thus, the sum of potential that each seed carries would be consistent. Making use of this trade-off was judged to be more effective in evaluating the competitive ability of the plant species than adjusting the number of seeds to a uniform value. To model the field environment, soil nutrient level was included as a factor potentially affecting the experimental results. Nutrient conditions simulated the edaphic environment of the cut slopes on which F. arundinacea is sown, and the surrounding environments into which it may spread.

Pot height and diameter were both 30 cm. Decomposed granite soil was laid on perlite with a 1:1 ratio. For seed germination and establishment, a 0.5 cm potting mix was spread. Seeds and soil mixture were scattered onto the topsoil. To simulate high nutrient levels, liquid fertiliser (6-10-5 NPK, HYPONeX JAPAN) was diluted at a ratio of 1:10,000, with 100 ml added per pot every 10 days. One month after this treatment, we could not find any difference between fertilised pots and sterile pots due to the poor nutrient-holding capacity of the granite-weathered residual soils. Therefore, 2 g solid fertiliser (11-8-7 NPK, HYPONeX KOREA) was applied monthly. No fertiliser treatment was applied to low-nutrient pots. Water was provided daily until germination and then supplemented when required. A total of 126 pots were used in the experiments, and pots were arranged by nutrient condition (Appendix E). The experiment ran from 9 April to 30 July 2018 in mesocosms set up at the glasshouse facility of biological sciences at Seoul National University. The mean temperature was  $23.5 \pm 3.7$  °C.

#### Measurements and data analysis

The period of measuring plant height and cover was dependant on plant growth rate. After the germination of the focal species, a week after sowing, the characteristics of early-stage plant growth were measured based on the percentage cover of a fixed area of the pot (Appendix F) and plant height (Appendix G) at intervals of 2 days. As growth accelerated in May, height and cover were measured every week. As growth slowed, height and plant cover were measured every 2 weeks from June onwards.

By mid-April (7 days after sowing), when the seedlings of *F. arundinacea* started to bud, the coverage of *Brassica napus* had already reached 40%. On 18 April (9 days after sowing), *Artemisia princeps, Aster yomena, B. napus, Cirsium setidens, Crepidiastrum sonchifolium* and *Lespedeza cuneata* 

were competitively dominant over *F. arundinacea*. After 18 April, the coverage of *C. sonchifolium* had increased dramatically. Overall, species belonging to FG 2 showed the fastest growth, and even grew faster than *F. arundinacea*. However, the species rank order of initial height was consistently maintained and did not change over time (Appendix G).

At harvest time (end July 2018), the number of shoots was counted, and aboveground biomass was collected at 1 cm height, dried at 75 °C for 72 hrs in a drying oven, and weighed.

The relative competition index (RCI) was used to interpret the competitive effect of neighbour species on *F. arundinacea*, and calculated using the following equation (Weigelt & Jolliffe, 2003):

$$RCI_{\gamma} = \frac{\gamma_{control} - \gamma_{treatment}}{\gamma_{control}}$$

 $\gamma_{control}$  refers to the performance of *F. arundinacea* in the monoculture pots and  $\gamma_{treatment}$  refers to the performance of *F. arundinacea* in either single- or multi-species pots with neighbours. A positive value indicates that the neighbour species suppressed the focal species, whereas a negative value indicates that the neighbour species facilitated the performance of the focal species. Values for RCI<sub>height</sub>, RCI<sub>cover</sub> and RCI<sub>biomass</sub> were calculated from plant height, plant cover and aboveground biomass. Values for RCI<sub>avg</sub> were derived from the mean of RCI<sub>height</sub>, RCI<sub>cover</sub> and RCI<sub>biomass</sub>, in view of their high correlation with each other.

The performance of *F. arundinacea* under different nutrient treatments was assessed by linear mixed model (LMMs) with pot as a random factor. Analysis of variance (ANOVA) was used to test the differences in performance of *F. arundinacea* under high- and low-nutrient conditions. When significant effects were detected, mean values were compared using the Scheffé test. Assumptions of normality of residuals and homoscedasticity were examined before performing ANOVA. Data were log-transformed if necessary, but when the assumptions were not met, the Welch correction or the Kruskal-Wallis test was applied with the Games-Howell post-hoc test. Whether RCI significantly differed from zero was verified using one sample *t*-test.

The effect of upper-layer plants on lower-layer plants in multi-species pots was examined to determine their interactions, for example, shading effects. Each plant in a multi-species pot was assigned a grade according to height (Appendix B). For example, *Lespedeza bicolour* was categorised as "High" owing to its rapid growth and tall stature, which enables it to shade plants at lower levels. A Kendall rank correlation test was performed to examine the relationships between plant relative coverage by layer. All statistical analysis was performed using R software Ver. 3.5.1 (R Core Team, 2008).

#### Results

The performance of *F. arundinacea* monoculture was generally poorer than that of pairwise pots and all multi-

species pots, resulting in RCI below zero. This implies that the density of the *F. arundinacea* monoculture pot may have been close to the threshold of severe intraspecific competition. In pairwise and multi-species competition experiments, faster growth and propagule density were found to mainly affect the performance of *F. arundinacea* when there was less intraspecific competition of *F. arundinacea*.

#### **Pairwise competition**

In pairwise tests, the relative competitive effects of neighbour species on *F. arundinacea* were dependant on functional group identity. Under nutrient-rich conditions, there was a significant difference in RCI<sub>avg</sub> between functional groups (log-transformed y, ANOVA, F = 17.3, p < 0.001; Fig. 1B). Functional group 2 showed the highest RCI<sub>avg</sub>, while values were negative for FG 1 and FG 3. Given the negative RCI<sub>avg</sub> values, species in FG 1 and FG 3 showed weak suppression effect, which prevented them from inhibiting the growth of *F. arundinacea* (p < 0.05, Scheffé test). Similarly, under the nutrient-poor condition, RCI<sub>avg</sub> for FG



**Fig 1.** Relative competition indices of pairwise combinations under (A) nutrient-poor and (B) nutrient-rich condition. FG 1 are perennial woody plants, FG 2 are annual forbs, and FG 3 are perennial plants. Uppercase letters represent significant differences between treatments (Scheffé test, p < 0.05). Lowercase letters represent significant differences between species within a functional group (Scheffé test, p < 0.001).



**Fig 2.** Competitive effect of species on *F. arundinacea* under (A) nutrient-poor and (B) nutrient-rich conditions in pairwise combinations. FG 1 are perennial woody plants, FG 2 are annual forbs, and FG 3 are perennial plants. Differences between  $\text{RCI}_{\text{avg}}$  and zero were verified by one-sample *t*-test. \*\*p < 0.01, \*p < 0.05.

2 was significantly different from that for FG 1 and FG 3 (ANOVA, F = 8.373, p < 0.01; Fig. 1A).

With respect to species performance within each FG, there were no differences within FG 1 (F = 4.404, p > 0.05) or FG 3 (F = 1.957, p > 0.05) under the nutrient-rich condition. However, in FG 2, *Brassica napus* and *Crepidiastrum sonchifolium* were significantly different from each other (F = 61.51, p < 0.01). Under the nutrient-poor condition, significant differences were observed in FG 1 (*Spiraea pru-nifolia, Albizia julibrissin* and *Lespedeza bicolour*) (F = 42.34, p < 0.001), but not in FG 2 (F = 2.606, p > 0.05). Significant differences also existed in FG 3 (F = 4.66, p < 0.01), where positive RCI<sub>avg</sub> values for *Dendranthema boreale* and *Artemisia princeps* contrasted with the negative values recorded for other species (p > 0.05, Scheffé test).

Under the nutrient-rich condition, *Brassica napus* (FG 2) showed a significant suppressive effect on *F. arundinacea* (p < 0.001, Scheffé test, Figs. 1B and 2B). In the low-nutrient treatment, the competitive ranking of *B. napus* was also significantly higher than that of any other species (p < 0.001, Scheffé test; Fig. 1A).

The RCI<sub>avg</sub> values for small-seeded plants such as *Spiraea prunifolia* and *Dendranthema boreale* were significantly higher under the nutrient-poor condition, indicating a



**Fig 3.** Relative competition indices of multi-species combinations under (A) nutrient-poor and (B) nutrient-rich conditions. Eight combinations can be found in Appendix D. Letters represent significant differences between treatments (Scheffé test, p < 0.05).

suppressive effect on *F. arundinacea* (p < 0.01, Scheffé test; Fig. 2A). Woody species such as *Lespedeza bicolour* and L. *cuneata* generally showed the lowest RCI<sub>avg</sub> values in both nutrient levels, indicating hardly any competitive effect on *F. arundinacea*.

#### Multi-species competition

Values of RCI<sub>avg</sub> were negative in both high- and lownutrient treatments in all multi-species experimental groups, indicating a weak competitive effect on *F. arundinacea*. However, the yielding effect on the growth of *F. arundinacea* under the nutrient-rich condition were significantly lower than under the nutrient-poor condition (ANOVA, F = 45.53, p < 0.001, Fig. 3).

Under the nutrient-rich condition, RCI<sub>avg</sub> was highest in Multi 7 (Artemisia princeps, Brassica napus, Lespedeza bicolour, Lespedeza cuneata) and lowest in Multi 3 (Crepidiastrum sonchifolium, L. bicolour, Metaplexis japonica, Pennisetum alopecuroides) and Multi 5 (Cirsium setidens,



**Fig. 4.** Competitive effect of species on *F. arundinacea* under (A) nutrient-poor and (B) nutrient-rich conditions in multi-species experiments. Differences between  $\text{RCI}_{avg}$  and zero were verified by one sample *t*-test. Eight combinations can be found in Appendix D. Letters represent significant differences between treatments (Scheffé test, p < 0.05). \*\*\*\* p < 0.001, \*\*p < 0.01, \*p < 0.05.

*C. sonchifolium, Dendranthema boreale*, L. *bicolour*), with the difference being statistically significant (F = 3.526, p < 0.05, Figs. 3B and 4B). Under the nutrient-poor condition, there were no significant differences amongst pots (F = 2.269, p > 0.05, Figs. 3A and 4A).

Taller and faster-growing plants affected the growth of smaller and slower-growing species. In general, the higher the coverage of upper-level species, the lower the coverage of underlying species in a pot under both nutrient conditions (Table 2, Appendix H). A significant negative correlation was found between cover of upper- and lower-level plants (Kendall's tau = -0.42, p < 0.001).

#### Nutrient treatments

In all experimental groups, the aboveground biomass of *F. arundinacea* was significantly affected by soil nutrient condition (LMMs,  $F_{1,104} = 390.11$ , p < 0.0001). The RCI<sub>avg</sub> calculated from the mean of RCI<sub>height</sub>, RCI<sub>cover</sub> and

**Table 2.** Coverage by species layer (Highest layer, Mid layer, Lowest layer). Eight multi-species combinations can be found in Appendix D. ANOVA or Welch ANOVA was performed depending on whether data met the assumptions of normality and homoscedasticity. Letters represent significant differences between levels (mean  $\pm$  SE, Tukey or Games-Howell test, p < 0.05).

	Multi 1	Multi 2	Multi 3	Multi 4	Multi 5	Multi 6	Multi 7	Multi 8
High Mid Low	$37.1 \pm 7.4^{a}$ $15.1 \pm 5.7^{b}$	$55.8 \pm 5.7^{a}$ $13.3 \pm 1.5^{b}$ $28.0 \pm 9.0^{b}$	$75.8 \pm 7.9^{a}$ $4.6 \pm 3.74^{b}$	$40.0 \pm 5.0$ $57.5 \pm 6.1$	$61.7 \pm 12.1^{a}$ $21.3 \pm 5.2^{b}$	$38.0 \pm 4.7^{a}$ $5.81 \pm 1.6^{b}$	$80.8 \pm 6.5^{ab}$ $83.8 \pm 3.9^{a}$ $15.9 \pm 10.2^{b}$	$85.4 \pm 3.9^{a}$ $16.8 \pm 5.3^{b}$

RCI<sub>biomass</sub> also differed significantly in relation to nutrient condition (LMMs,  $F_{1,104} = 159.8$ , p < 0.0001).

#### Discussion

## Plant performances by functional group identity

While intraspecific competition in monocultures was severe, resulting in difficulty in measuring species interactions under different plant combinations, invasion-resistance was found to be partly predictable from functional group identity. Functional groups were defined using specific traits such as faster growth that could be effective in conferring biotic resistance. However, the functional combinations did not successfully prevent the growth of *Festuca arundinacea*, contrary to the predictions of the limiting similarity and diversity-resistance hypotheses.

In pairwise competition experiments, FG 2 showed the most significant inhibitory effect on the performance of the focal species in comparison to the other functional groups. In particular, the remarkable performance of *Brassica napus* in FG 2 may be attributable to the characteristics of this annual herb. The rapid growth of annual herbs suggests a niche preemption strategy (Byun & Lee, 2017; Byun et al., 2013; Svejcar & Sheley, 2001), involving the depletion of nutrients required by other slower-growing species in the community (Fukami, Martijn Bezemer, Mortimer, & Putten, 2005; Mwangi et al., 2007; Svejcar, 1990). This suggests that niche preemption was more important than the limiting similarity effect in allowing the neighbour species to gain a competitive advantage over *F. arundinacea*.

Considering the species showing the lowest RCI<sub>avg</sub> in the pairwise experiments, woody species may have had a yielding effect on the growth of *F. arundinacea. Festuca arundinacea*, a member of the family Poaceae, forms a short rhizome spreading near the soil surface, whereas *Lespedeza bicolour*, a common legume shrub, has a much deeper rooting profile. Shrub species typically have deeper roots than herbaceous species (Jackson et al., 1996). Thus, these two species may not experience severe belowground competition. Previous research also suggests that grasses may have an advantage over shrubs (D'Antonio et al., 1992). Similarly, we suggest that woody species may avoid direct competition with other herbs and grasses in a pot by root extension and nutrient absorption from deeper in the soil.

By contrast, small-seeded plant species reduced the growth of the focal grass species under the nutrient-poor condition by increasing propagule pressure. Density suppression by small-seeded species has been observed in previous studies (Moles & Westoby, 2004; Yannelli, Hughes, & Kollmann, 2017). Small and slow-growing plants may compete with *F. arundinacea* belowground for the same rooting area, an example of functionally similar species occupying similar niches, with consequently higher interspecific competition (Macarthur & Levins, 1967; Yannelli et al.,

2017). In our experiment, although seed mass was included as a trait in functional group definition, there was wide within-group variation in this parameter. Functional groups were not differentiated by seed mass, but the effect of small seed mass and high density was revealed by species identity.

The limiting similarity hypothesis predicted that F. arundinacea would be suppressed by species in the same functional group exploiting similar resources. However, Brassica napus in FG 2 was effectively suppressive on F. arundinacea, and the competitiveness of plants in the same functional group as F. arundinacea (FG 3) was generally not as high as for the other groups. Therefore, we conclude that the FG 3 functional traits were not highly correlated with competitive ability. There was disparity in species performance within a functional group, but the main defining characteristics such as seed mass, dormancy form and longevity were important influences on the competitiveness of native species relative to the focal species in their early stages (Byun et al., 2017; Funk et al., 2008; Mwangi et al., 2007). Plant species contributing to within-group disparity showed characteristics that did not match the overall identity of the group. For example, Spiraea prunifolia in FG 1 is a woody species, as are others in FG 1, but unlike them, it has small seeds. However, groups comprising generally similar traits may yield completely different results if the detailed traits of member species are dissimilar (Burns & Winn, 2006; Funk & Vitousek, 2007). If functional groups were primarily defined by seed mass, the results of this study would be interpreted differently. One of the challenges of this approach is the identification of relevant and measurable functional traits (Funk et al., 2008; Funk et al., 2017; Price & Pärtel, 2013), which is necessary to increase predictive power. We consider that our functional groups were valid since the constituent species were similar in most characteristics, but that seed mass was an especially critical trait for competitive ability in the colonisation stage of the grass species. Moreover, niche preemption and woodiness should also be examined in long-term observation experiments. This study covered only a short period of colonisation; thus, potential growth phase-related variations in plant functional traits and biological interactions would have been overlooked. There was an unbalance in the number of species per functional group because we tried to use native species that passed the pre-germination test; thus, further examination is required.

#### Density pressure and biotic resistance

The results from multi-species experimental groups were not consistent with those of the pairwise competition experiments. We used the invasion-resistance model to design the experiments with the aim of creating functional redundancy and inducing niche overlap, but the suppressive effect on *F*. *arundinacea* was marginal. Moreover, some effects on growth of *F. arundinacea* that were observed in the pairwise experiments were confounded by other interactions in multispecies pots. It appears that the combination of several native species evoked different results by species interactions rather than exhibiting functional redundancy.

We recorded a density suppression effect on F. arundinacea. Particularly different from the results of previous studies (e.g., Byun et al., 2013) was that seed mass per pot was uniformly adjusted in the present study, resulting in differences in density amongst pots. In monoculture, pairwise and multi-species pots, the numbers of F. arundinacea and neighbour species were not identical, leading to different intensities of intra- and interspecific competition in each pot. Compared with monoculture pots, the density of the focal species in pairwise pots was 1/2 and 1/5 of those in multispecies treatments. The fact that the overall performance ranking of F. arundinacea was found to be monoculture<pairwise<multi-species could mean that F. arundinacea was mainly controlled by intraspecific competition in this setting. In general, the coexistence of species in a community implies that intraspecific competition>interspecific competition (Adler et al., 2018; Chesson, 2000), but the density of the monocultures in this study may have been close to the threshold controlling the growth of F. arundinacea. The performance of F. arundinacea improved in the pairwise pots as density of the species decreased. Aside from the niche preemption effect, its performance decreased in intense competition with small-seeded species and increased in the pots with woody species. In the latter case, niche partitioning resulted in a decrease in density suppression. Achieving biotic resistance by seed density has been reported widely, and our results, which underline the importance of seed density rather than functional group combinations, were similar to those of Byun et al. (2015) and Yannelli et al. (2018).

The observed interactions amongst species in multispecies pots caused results to differ from those in the pairwise experiment. The suppressive effect of annual plants, whose strategy involves the rapid preemption of resources, may have been modified by the combined presence of shrub species. A structurally-complex community is likely to create more spatial niches (Davis, Grime, & Thompson, 2000; Palmer et al., 1997), and shrub species in multi-competing pots might therefore provide microcracks for focal species by placing their roots at different depths. As most multi-species combinations included species from each functional group, shrubs belonging to FG 1 may have allowed the growth of *F. arundinacea* rather than impeding it.

Although we did not design the experiment to test shading effect, results from multi-species pots also showed that tall, fast-growing species inhibited the growth of lower-layer vegetation by shading. This can be considered a "higherorder" interaction, referring to the indirect effect that occurs when one species modifies the interaction between two other species (Wootton, 1994a; 1994b). Higher-order interactions were intensified in the nutrient-rich condition, as the available nutrients were taken up disproportionately by the fast-growing species. Thus, as the results showed, lower plants faded away with their rapid growth. Shading may reduce the density-suppression effect of small-seeded species. Still, as mentioned earlier, this experiment was not designed for the purpose of verifying the shading effect, and it should be noted that in the case of short plants, sunlight coming from the side may have hit them. Although a higher biotic resistance of multi-species combinations was predicted, based on the assumption of niche complementarity by diverse traits, it was not fully expressed due to the abovementioned higher-order interactions.

## Nutrients and biotic resistance

Although resource competition is one of the main drivers of plant community assembly (Grime, 1973; Newman, 1973), and is driven by the differing resource exploitation strategies of plant species (Violle et al., 2007), few studies have considered both functional traits and nutrient levels. In our study, nutrients did not significantly affect the results in pairwise pots, but in the multi-species experiment, they did influence the effect of neighbour species on the performance of F. arundinacea. In nutrient-rich environments, faster-growing species and woody species influenced the slow-growing species, reinforcing the overall pattern found in multi-species pots. High or low nutrient levels determined the performance of plants by functional group. Similar results were reported by (La Pierre & Smith, 2015), where functional traits of species preferring the nutrient-rich condition drove shifts in a grass community over a period of several years. When designing a plant community to promote biotic resistance, both functional group identity and nutrient level should be used to predict the higher-order interactions amongst species.

## Conclusion

Overall, we conclude that the functional group approach was not sufficient to promote biotic resistance to the invasion of *F. arundinacea*. However, density suppression did effectively inhibit the growth of this grass. The competitive relationships between native plants and *F. arundinacea* were partially dependant on functional group identity but were inconsistent with the limiting similarity hypothesis and the diversity-resistance hypothesis. The hierarchy of species characteristics is an important influence on community structure. Therefore, it is necessary to consider interspecific interactions when predicting community-level species dynamics. Nutrient condition did not change the competitive rank order of species but reinforced the growth of both faster-growing and shading woody plant species, resulting in a density-suppressive effect.

## **Declaration of Competing Interest**

Not applicable

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# **Supplementary materials**

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