Journal of Ecology

British Ecological Society

Journal of Ecology 2011, **99**, 1308–1315 doi: 10.1111/j.1365-2745.2011.01858.x

Comparison of nutrient acquisition in exotic plant species and congeneric natives

Annelein Meisner[1]*, Wietse de Boer[2], Koen J. F. Verhoeven[1,3], Henricus T.S. Boschker[4] and Wim H. van der Putten[1,3]

[1]*Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), PO Box 50, 6700 AB Wageningen, The Netherlands;* [2]*Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), PO Box 50, 6700 AB Wageningen, The Netherlands;* [3]*Laboratory of Nematology, Wageningen University, PO Box 8123, 6700 ES Wageningen, The Netherlands; and* [4]*Department of Marine Microbiology, Netherlands Institute of Ecology (NIOO-KNAW), PO Box 140, 4400AV Yerseke, The Netherlands*

Summary

1. The ability of exotic plant species to establish and expand in new areas may be enhanced by a relatively high ability to acquire soil nutrients. To test this hypothesis, we predicted that the capacity for nutrient acquisition would be higher in seedlings of exotic species than in seedlings of native congeners.

2. We selected the five exotic species that had recently increased in abundance in a riverine habitat in the Netherlands and that had a native congener that was common in the same habitat. We grew seedlings of each of these ten species singly in pots of soil from this habitat in a glasshouse. After two months, we measured the final dry mass and N and P content of each plant and components of microbial biomass and nutrient mineralization in the soil. We also measured these soil characteristics in pots that had been left unplanted.

3. Exotic and native congeners did not differ consistently in the uptake of N or P or in effects on components of soil mineralization. Within a genus, values of these measurements were sometimes higher, sometimes lower and sometimes similar to the exotic when compared with the native species.

4. Depending upon the statistical analysis used, biomarker-based biomass of arbuscular mycorrhizal fungi was generally higher in soil planted with exotic than with native species. Most measures of microbial biomass and soil mineralization were higher in pots that had been planted with plants than in pots with no plant.

5. *Synthesis.* Our results do not suggest that invasive, exotic plant species generally possess greater capacity for nutrient acquisition during the early establishment than native species do.

Key-words: invasion ecology, nitrogen mineralization, phosphatase activity, plant nutrient uptake, plant range expansion, plant–soil (below-ground) interaction, PLFA, rhizosphere, soil microbial community structure

Introduction

Over many years, humans have, both intentionally and accidentally, introduced a large number of plant species into non-native habitats across much of the biosphere (Hodkinson & Thompson 1997; Vitousek *et al.* 1997). Only a small number of these introduced exotic plant species are able to establish in their new region, and only a subset of those species will spread into new areas (Williamson & Fitter 1996; Richardson *et al.* 2000). Several exotic plant species have been shown to increase nutrient cycling after establishment (e.g. Duda *et al.* 2003; Chapuis-Lardy *et al.* 2006; Sharma & Raghubanshi 2009), especially when natives were displaced by plant species from different functional groups (Ehrenfeld 2003; Liao *et al.* 2008). The impact of exotic plant species on ecosystem processes is mostly studied via comparisons of invaded and non-invaded sites. This approach has the advantage in that it includes long-term changes in litter and nutrient dynamics, but this does not indicate whether altered nutrient dynamics play a role during the establishment of exotics.

Exotic plant species may be expected to exploit soil nutrients better than natives do, given that their above-ground nutrient concentrations are often higher and resource use strategies are often more efficient than those of native species present in the

*Correspondence author. E-mail: a.meisner@nioo.knaw.nl

invaded range (e.g. Agrawal *et al*. 2005; Funk & Vitousek 2007). Furthermore, exotic plant species are often observed to be released from natural enemies such as pathogens and herbivores (Keane & Crawley 2002; Klironomos 2002). For example, two recent studies observed that exotic plant species that invaded a riverine habitat experienced less negative effects from soil biota (Van Grunsven *et al*. 2007; Engelkes *et al*. 2008). In addition, exotic plant species, which could be released from enemies in their new range, have been observed to allocate more of their resources to plant production instead of defence compared with the species from the native range (Blair & Wolfe 2004; Zou *et al*. 2006; Feng *et al*. 2009), possibly as a result of increased uptake of soil nutrients in the new range (Zou *et al*. 2006).

Increased nutrient uptake may be accomplished by increased root growth, stimulation of microbial decomposers via rhizodepositon or allocation of carbon to arbuscular mycorrhizal fungi (AMF) (Hobbie 1992; Aerts & Chapin 2000; Chapin, Matson & Mooney 2002). Stimulation of biomass and activity of microbes may enhance plant growth via increased mineralization of nutrients in the soil organic matter (e.g. Kuzyakov, Friedel & Stahr 2000; Dijkstra *et al*. 2009). Moreover, nutrients will become released when rhizosphere microbes are grazed by soil fauna (Clarholm 1985; Ingham *et al*. 1985; Bonkowski, Villenave & Griffiths 2009). Plant phosphorus nutrition can also be enhanced by symbiosis with AMF, providing phosphorus to the plants in return for carbon (Smith & Read 2008). However, AMF can also 'cheat' by being parasitic instead of mutualistic to plant species (Johnson, Graham & Smith 1997; Klironomos 2003; Kiers & van der Heijden 2006).

Here we compare nutrient acquisition strategies of exotic plant species and phylogenetically related native congeners during their establishment phase to test the hypothesis that invasive exotic plants have relatively high nutrient acquisition during early establishment. We specifically predicted that exotic plant species would show greater uptake of N and P than native congeners. We further predicted that exotics had lower amounts of available phosphorus, greater fluxes of N, P and biomass of microbes in the rhizosphere than natives. We also expected that the uptake of N would be correlated with fluxes of N in soil and that the uptake of P would be correlated with both P mineralization and AMF biomass. We tested these predictions in a glasshouse experiment using five exotic and five phylogenetically related native plant species all co-occurring in the same habitat. This approach enabled us to control for phylogenetic differences between native and exotic plant species (Harvey & Purvis 1991), as well as for habitat-specific aspects, including physical, chemical and biological soil characteristics.

Materials and methods

PLANTS

Five exotic plant species and their five native congeners, all members of the Asteraceae, were selected for the experiment with the following selection criteria also used by Engelkes *et al*. (2008): (i) exotic plant species increased in frequency in the second half of the 20th century; (ii) a native plant species within the same genus had to be present in the Netherlands; (iii) the native plant species is abundant in the Netherlands; (iv) the native and exotic plant species within a genus co-occur within the same habitat; (v) seeds that can be germinated had to be present for both native and exotic plant species; and (vi) all plant pairs had to be present in the same habitat. There were five pairs that fulfilled these criteria (Table 1). They all occur in the Geldersche Poort region (51°52′ N; 6°02′ E) along the river Waal (Dirkse, Hochstenbach & Reijerse 2007).

SEED COLLECTION AND SEEDLING GERMINATION

Most of the plant seeds were collected along the river Waal (*Artemisia biennis, Artemisia vulgaris*, *Bidens tripartita, Senecio inaequidens*, *Senecio vulgaris* and *Tragopogon pratensis* subsp. *pratensis)*. Seeds of *Tragopogon dubius* were collected in the centre of the Netherlands (52°16′ N; 5°35′ E), whereas seeds of *Bidens frondosa, Centaurea jacea* and *Centaurea stoebe* were obtained from small seed companies that collect seeds locally in the Netherlands or Germany.

Seeds were surface-sterilized in a 0.5% sodium hypochlorite solution, germinated on glass beads, moistened with demineralized water and placed in a germination cabinet. As not all seeds germinated at the same time, seedlings were placed in a 4 °C climate chamber with 10 h light per 24 h after germination until the start of the experiment. Seedlings from *B. frondosa* and *B. tripartita* were kept in sterilized soil after germination, because these seedlings did not survive on glass beads.

SOIL SAMPLING AND PREPARING

In September 2007, soil was collected from five locations in the nature reserve Millingerwaard (51°52′ N; 6°00′ E), which is part of the Geldersche Poort region in the Netherlands. All plant species used for our experiment occur in this region and most of them co-occur in the Millingerwaard. This nature reserve is located along the river Waal, which is the southernmost branch of the river Rhine. The Millingerwaard, as other nature reserves in this region, is grazed by horses and cattle and flooded periodically during high water discharge of the river. After sampling, the soil was homogenized, sieved through a

Table 1. Exotic and native plant species selected for the experiment. Plant species names follow Van der Meijden (2005). Bold plant names are exotic plant species

Plant Species	Origin*,†,‡	Time of first introduction*,†,‡
Artemisia vulgaris	Native¶	
Artemisia biennis	**North Asia**	**1950–75**
Centaurea jacea	Native¶	
Centaurea stoebe	**Mid-Europe**	**1950–75**
Tragopogon pratensis ssp. *pratensis*	Native¶	
Tragopogon dubius	**Mid- and South Europe**	**1950s**
Bidens tripartita	Native¶	
Bidens frondosa	**North America**	**1929**
Senecio vulgaris	Native¶	
Senecio inaequidens	**South Africa**	**1925–50**

*Tamis *et al*. 2005, †Weeda *et al*. 1991, ‡Dirkse, Hochstenbach & Reijerse 2007, ¶Native to the Netherlands.

10-mm mesh, and stored outdoors for 2.5 weeks. The sandy soil (90.5% sand, 9.47% silt and 0.09% clay) contained 14% moisture (w/w), had a pH_{water} of 7.94, 1.97% C and a C:N ratio of 20.3.

EXPERIMENTAL DESIGN AND SET UP

In our experimental design, we had five exotic and five phylogenetically related native plant species. There were 10 replicate pots for each plant species and 10 control pots without plants. The control pots had been set up at the same time as the pots with plants and were included to determine the effects of plant presence on soil nutrient cycling, soil nutrient pools and microbial community structure. Before planting the seedlings, 3-L pots were filled with moist field soil equivalent to 2500 g dry soil per pot. During the experiment, all soil were kept at 50% water holding capacity, which is equivalent to17.7% moisture based on dry soil. Each pot was planted with seedlings from one of the five exotic plant species or their native congeners. Three seedlings were planted per pot. We planted two individuals per pot for both native and exotic plant species within *Tragopogon* and *Bidens* pairs, because seedling availability was limited. The plants were grown in a climate-controlled glasshouse with a day temperature of 21 °C (±2 °C) and a night temperature of 16 °C (±1 °C). There was 16 h daylight in the greenhouse and artificial light was supplemented up to 225 μmol m^{-2} s^{-1} when natural daylight fell below this level. The experiment was started on 16 October 2007 and the plants were harvested on 10 December 2007. At harvest, soil, roots and shoots were separated.

PLANT BIOMASS, NUTRIENT CONTENT AND SOIL ANALYSES

The harvested plant material was dried at 70 °C to determine shoot and root biomass. The dried roots and shoots were ground and analysed for total C, N and P content. Total C and N were determined in 3 mg of plant material using an NC analyser (Thermo flash EA 1112, Thermo Scientific, Milan, Italy), which uses flash combustion in conjunction with an analytical column and a thermal detector to determine percentages of N and C in the plant samples. Total P was measured following digestion with a mixture of H_2SO_4-Se and salicylic acid (Novozamsky *et al.* 1984). Briefly, digestion was performed by incubating a mixture of 300 mg plant material and 2.5 mL of a mixture of H_2SO_4-Se and salicylic acid for 3 h at 100 °C, 20 min at 150 °C and finally at 325 °C until liquid was transparent. Then, 48.3 mL Milli-q water was added and the mixture was filtered. Phosphorus in the filtrate was coloured with Molybdate reagens, and was measured against a reference line on a plate reader (bio-tek synergy HT-1, biotek, Winooski, USA) at 720 nm. With these data, the total nutrients in the plant were calculated for N and P separately using the following formula: Total N and P = (shoot concentration × shoot biomass) + (root concentration × root biomass).

Soil sub-samples were stored in plastic bags at 4 °C prior to analysis. Soil water content was determined as weight loss after overnight incubation at 105 °C. All soils reached constant mass after overnight incubation. This dry weight was used to calculate soil nutrient concentrations and mineralization activities.

Potential ammonification activity was determined using the arginine ammonification assay according to Alef & Kleiner (1987) with minor modifications. For each pot, two replicate samples of fresh soil (equivalent to 1 g dry weight) were spiked with 0.25 mL arginine solution (4.8 mg arginine per mL milli-q water), and one control was left unspiked. After 3 h of incubation at 25 °C, the reaction was stopped by adding 4 mL 2 M KCl. Ammonium was extracted by shaking for 15 min. Arginine was also added to control for background colour. Samples were centrifuged at 5250 × g for 10 min and dilutions (1:1 with water) were stored at −20 °C until analyses. Potential nitrifying activity was measured as accumulation of nitrite and nitrate over a 3-h period in ammonium-enriched soil slurries as described by Bodelier *et al.* (1998). N-NH_4^+ and N-NO_3^- concentrations were measured on a Technicon TrAAcs 800 autoanalyser (Technicon Instruments Corp., Tarrytown, NY, USA).

Alkaline phosphomonoesterase is produced by soil microorganisms. This extra-cellular enzyme releases phosphate from phosphomonoesters in soil organic matter. The phosphate released from soil organic matter by alkaline phosphomonoesterase can be taken up by plants. Enzyme activity was measured according to Schinner *et al.* (1995) with the following modifications: two samples and one control were centrifuged at 5250 × g for 10 min and the supernatant was diluted five times before the released *p*-nitrophenol was measured on a plate reader (biotek synergy HT-1, biotek, Winooski, VT, USA) at 405 nm.

Potential plant-available inorganic P was estimated via extraction of soil subsamples in 0.5 M $NaHCO_3$ (P_{olsen}) (Troelstra, Wagenaar & De Boer 1990). To measure P_{olsen}, soil sub-samples were dried at a maximum of 40 °C for 1 week after harvest.

MICROBIAL BIOMASS

Phospholipid derived fatty acids (PLFA's) were extracted from freeze-dried soil samples with a three-step extraction protocol according to Boschker (2004). This consisted of a Bligh and Dyer total lipid extraction, a fractioning of the total lipids on a silicic-acid column with chloroform, acetone and methanol, and finally mild-alkaline derivation to release the fatty acid methyl esters (FAMEs). Neutral lipids were collected in the chloroform fraction, and phospholipids were collected in the methanol fraction. The FAMEs were analysed using gas chromatography–flame ionization detector (GC–FID) on a Focus GC (Thermo Scientific, Bremen, Germany) with a Zebron ZB5 column (dimensions: 60 m, 0.32 mm, 0.25 μm; Phenomenex, Torrance, CA, USA). Peak areas were calculated relative to an internal standard (19:0). In total, 28 different PLFA's were detected and identified based on retention time comparisons with FAME standards. The following PLFA's were chosen to represent bacterial biomass: i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0, 17:0, cy17:0, 18:1ω7 and cy19:0 (Frostegård & Bååth 1996); total fungal biomass: 18:2ω6 (Federle 1986); actinomycetes: 10Me16:0, 10Me17:0, 10Me18:0 (Frostegård, Tunlid & Bååth 1993). Fungal: bacterial ratios were based on 18:2ω6:bacterial PLFA ratio (Frostegård & Bååth 1996). The PLFA-based AMF biomarker 16:1ω5 was not a good indicator for arbuscular mycorrhizal fungal biomass, because this biomarker is also present in bacteria (Olsson 1999; Hedlund 2002). Therefore, the neutral lipid fatty acid (NLFA) 16:1ω5 was used as an AMF biomarker (Hedlund 2002). Ergosterol was also used to measure fungal biomass. This is a specific fungal biomarker in the cell wall of fungi, but not in AMF (Olsson *et al.* 2003). This was extracted from frozen soil using an alkaline-extraction method and measured on a HPLC (Dionex, Germering, Germany) equipped with a C 18 reverse-phase column (Alltech, Deerfield, IL, USA) and a UV-detector set at 282 nm (De Ridder-Duine *et al.* 2006).

DATA ANALYSES

Fixed-effect models with origin, genus and their interaction were performed using Statistica version 8 (Statsoft, Inc. (2007), Tulsa, OK, USA), following the approach of previous studies with similar

experimental design (e.g. Agrawal *et al*. 2005). Planned comparisons were used to test for differences between species within genera. Genus was considered fixed, because plant species were selected non-randomly following the list of criteria described above, and we included all genera that met these criteria. A consequence of this approach is that the inference space for the statistical test is limited to the genera and plant species included in the experiment and does not extend to other genera that were not included in our experiment. As experimental designs similar to ours are sometimes analysed differently, we also fitted models with genus as random factor (using the type III ANOVA implementation of PROC MIXED in SAS for windows 9.1., the SAS institute, Cary, NC, USA). A drawback of this approach is that it is underpowered with only few levels (five in our study) of the random factor included in the study (Bart, Fligner & Notz 1998). Therefore, the random factor approach is presented in the Table S1 in Supporting Information, and is briefly discussed in the result section.

Data were transformed when necessary to meet the assumptions for ANOVA. The following transformations were used: y^2 for total plant N uptake; ln(y) for total plant P uptake, potential nitrifying activity and fungal biomass (PLFA biomarker); $y^{(-1/2)}$ for nitrate concentration in soil and biomass of actinomycetes in soil; and $y^{(-1/4)}$ for fungal:bacterial ratio in soil. Control pots and planted pots were analysed with planned comparisons after a one-way ANOVA at pot level (see Tables S2 and S3). Pearson correlations between plant nutrient uptake and soil nutrient mineralization measurements were performed. For each table of results presented in the manuscript, we set a table-wide false discovery rate (FDR) threshold for significance at FDR = 0.05 to control for inflation of type I error due to multiple testing (Benjamini & Hochberg 1995; Verhoeven, Simonsen & McIntyre 2005). FDR is typically more powerful than Bonferroni approaches and has fewer false negative results (= type II errors) (Verhoeven, Simonsen & McIntyre 2005). The FDR method evaluates ranked *P*-values against a rank-specific significance threshold, and results in a list of significant *P*-values of which a pre-determined proportion are false discoveries. Thus, at FDR = 0.05, 5% of the significant results are expected to be false discoveries (= type I error).

Results

PLANT NUTRIENT UPTAKE

There was no consistent difference in nutrient uptake between exotic and native congeners (Table 2; Fig. 1). Therefore, the results did not support the hypothesis that exotic plant species exploit soil nutrient pools better than congeneric native plant species. There was also no significant origin effect for total C content in plant species if analysed with genus as a random factor (Table S1), and lower C content in exotic plant species was only observed within three genera (Fig. S1). However, total C

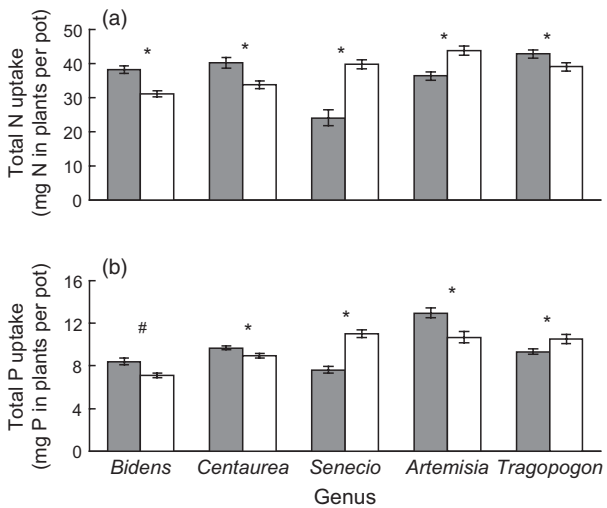

Fig. 1. (a) Total N uptake and (b) total P uptake. Mean ± SE are presented for native (grey bars) and exotic (white bars) plant species. * indicate $P < 0.05$ and # indicates $P < 0.1$ for pair wise within-genus comparisons.

Table 2. Comparison of genus and origin differences for nutrient acquisition, microbial activity and microbial biomass using a two-way fixed-factor ANOVA. Bold *P*-values indicate that they are significant at a false discovery rate threshold of $P = 0.05$ (see Materials and methods)

		Genus		Origin		Genus × Origin	
	d.f.$_{den*}$	*F*	*P*	*F*	*P*	*F*	*P*
Total N uptake	86	16.3	**< 0.001**	1.74	0.19	29.4	**< 0.001**
Total P uptake	84	38.2	**< 0.001**	0.06	0.95	21.5	**< 0.001**
Total C in plant	85	2.74	0.033	8.73	**0.004**	48.0	**< 0.001**
Total biomass	88	1.06	0.38	5.51	0.02	34.2	**< 0.001**
Arginine ammonification	87	9.76	**< 0.001**	2.78	0.099	2.84	0.03
Potential nitrifying activity	82	1.13	0.27	1.72	0.19	1.29	0.28
Phosphomonoesterase	88	81.9	**< 0.001**	14.8	**< 0.001**	1.87	0.12
P_{olsen}	88	4.72	**0.002**	2.38	0.13	0.62	0.65
Microbial biomass	84	0.05	0.99	0.27	0.6	1.5	0.21
Bacterial biomass	84	0.04	1	0.05	0.84	1.14	0.43
Fungal biomass (PLFA)	84	2.46	0.051	6.92	**0.01**	3.03	0.02
Fungal biomass (ergosterol)	77	2.74	0.04	1.55	0.22	1.31	0.27
Actinomycetes	84	0.65	0.63	0.02	0.90	1.45	0.22
Fungi : bacteria (PLFA)	84	4.04	**0.005**	11.4	**0.001**	2.39	0.06
AMF	86	14.62	**< 0.001**	7.91	**0.006**	6.40	**< 0.001**

*d.f.$_{den}$ is denominator degrees of freedom (d.f.). numerator d.f.: genus = 4; origin = 1; genus × origin = 4.

content was lower in exotic than native congeners if analysed with genus as a fixed factor (Table 2 and Fig. S1).

SOIL NUTRIENT FLUXES

No origin effects were observed for N and P fluxes if genus was considered a random factor and multiple testing correction was performed (Table S1). However, alkaline phosphomonoesterase activity was significantly higher in soil from exotic plant species than in soil from native congeners if tested with genus as a random effect without correction for multiple tests or with genus as a fixed effect (Table 2 and Fig. 2). The significant effect of origin was mainly due to differences within two genera. Origin did not affect concentration of plant-available P (P_{olsen}; Table 2 and Fig. S2) or fluxes of N as measured by arginine ammonification and potential nitrification (see Table 2 and Fig. 2). Pots with plants had higher ammonification and lower nitrification than unplanted pots and generally had lower rates of alkaline phophomonoesterase (Table S2).

MICROBIAL BIOMASS IN SOIL

Origin did not affect mass of soil microbes when genus was treated as a random factor (Table S1). However, PLFA-based fungal biomass and NLFA-based AMF biomass were on average higher in soil of exotic plant species than in that of native plant species if the data were analysed with a fixed-factor approach (Table 2, Fig. 3 and Fig. S3). Microbial biomass tended to be higher in pots with plants than in unplanted pots (Table S3).

CORRELATIONS BETWEEN PLANT NUTRIENT UPTAKE AND SOIL NUTRIENT FLUXES AND AMF

Plant N uptake did not correlate with soil arginine ammonification or nitrifying activity ($P > 0.05$). Plant P uptake did not correlate with NLFA-based AMF biomass or plant-available P concentrations. However, alkaline phosphomomoesterase activity correlated negatively with P uptake ($r = -0.48$, $P < 0.001$).

Discussion

Overall, our results do not support the hypothesis that exotic plant species are generally better able to exploit soil nutrient pools during their establishment than native plant species. Instead, in our study, rhizosphere nutrient fluxes and plant N and P uptake were higher, lower or did not differ between exotic and native plant species within the studied genera. These results are consistent with a study of effects of two exotic and

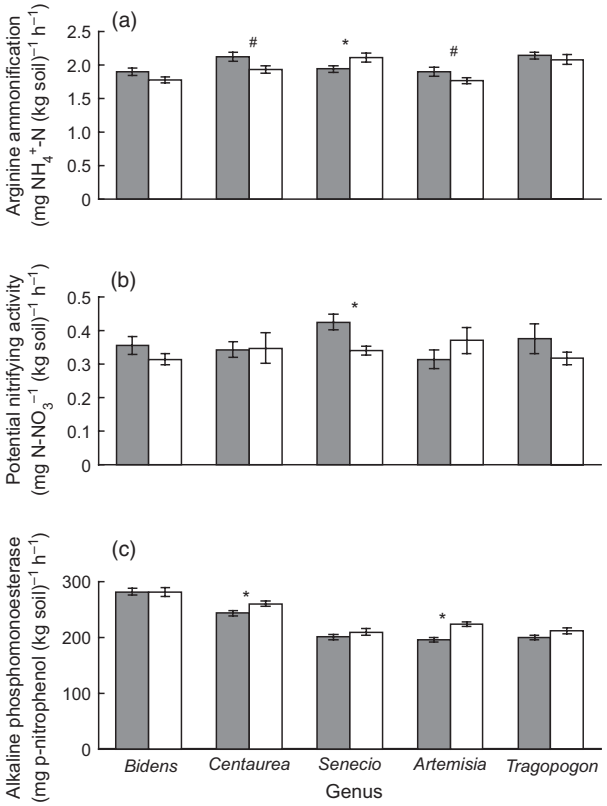

Fig. 2. (a) Arginine ammonification, (b) potential nitrifying activity and (c) alkaline phosphomonosterase activity in soil. Mean ± SE are presented for native (grey bars) and exotic (white bars) plant species. * indicates $P < 0.05$ and # indicates $P < 0.1$ for pairwise within-genus comparisons.

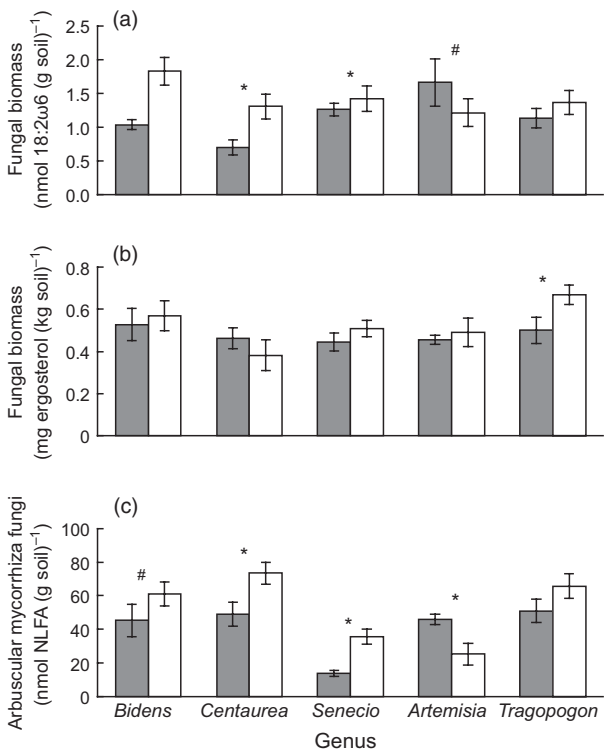

Fig. 3. (a) fungal biomass measured with PLFA biomarker 18:2ω6, (b) fungal biomass measured as ergosterol concentration and (c) AMF biomass measured with the NLFA C16:1ω5 biomarker. Mean ± SE are presented for native (grey bars) and exotic (white bars) plant species. * indicates $P < 0.05$ and # indicates $P < 0.1$ for pairwise within-genus comparisons.

one native plant species in the US on short-term N mineralization (Kourtev, Ehrenfeld & Haggblom 2003).

Plant presence increased arginine ammonification and microbial biomass, but decreased nitrification rates in comparison with control pots. This plant-induced stimulation of N-mineralizing microbes and simultaneous suppression of nitrifying bacteria points at an increased N flux from soil organic matter, whereas soil conditions remain N-limited. Under such conditions plants are often better competitors for $N\text{-}NH_4^+$ than nitrifying bacteria (Verhagen *et al*. 1994). However, plant N uptake did not correlate with potential soil N mineralization, possibly because the range of arginine ammonification rates between plant species was too narrow. Furthermore, plant species may have used additional strategies for N uptake to the ones we have measured here. For example, the turnover of soil microbes, e.g. via soil fauna, may have released N for plant uptake when they were grazing on rhizosphere microbes (Clarholm 1985; Ingham *et al*. 1985; Bardgett & Chan 1999; Bonkowski 2004).

Phosphorous uptake differed between some species within genera. However, it is not clear which processes caused this difference, because a higher P uptake did not correlate with a decrease in potential plant-available inorganic P pools (P_{olsen}) or higher organic P mineralization as indicated by alkaline phosphomonoesterase activity. Interestingly, native plant species inhibited alkaline phosphomonoesterase more than exotic plant species did, at least when analysed with genus as a fixed effect. Alkaline phosphomonoesterase is excreted by soil microbes in situations with low P availability (Bardgett 2005). However, our soil was not P-limited, because the concentrations of potential plant-available P (P_{olsen}) in our study is considered to be moderately high (26–45 mg P per kg soil; Sharpley & Withers 1994). Furthermore, the low N:P ratios in plant shoots (range N:P: 3.1 ± 0.3–8.0 ± 0.3; data not shown) indicated that our plant species were N-limited rather than P-limited (Koerselman & Meuleman 1996).

Exotic plant species in our study had on average more NLFA-based AMF biomass than congeneric native plant species when analysed with genus as a fixed effect, except for the genus *Artemisia,* where an opposite pattern was observed. In other studies, exotic plant species have been observed to increase (e.g. Walling & Zabinski 2004; Batten *et al*. 2006) or decrease AMF-abundance (e.g. Mummey & Rillig 2006; Vogelsang & Bever 2009). Those studies used a variety of different techniques to quantify AMF-abundance. Here we measured AMF with the NLFA-biomarker, which is present in storage lipids in external hyphae (Olsson 1999; Hedlund 2002). Therefore, exotic plant species in our study might have a higher carbon allocation to AMF hyphae than native plant species, but without a higher uptake of P. It is possible that a reduction in the dependence on AMF of the exotic plant species studied here (Seifert, Bever & Maron 2009) might have contributed to the observed results (Pringle *et al*. 2009) and made the AMF more parasitic to the exotic plants (Bever 2002). Interestingly, our results suggest that there was no origin effect for saprotrophic fungal biomass when using ergosterol (Fig. 3). It could be that fungal biomass in soil needs more time to respond, because a study on exotic *Solidago gigantea* showed that it takes more than one growing season to increase fungal biomass (Scharfy *et al*. 2010).

One of the key questions of this study was to determine if, as cause for their establishment, exotic plant species would exploit soil nutrients better than natives. At establishment, there was no pattern in our study indicating that exotic plant species would differ consistently in nutrient acquisition from native congeners. However, previous studies have shown that exotic plant species experience a less negative effect from soil biota than native congeners (Van Grunsven *et al*. 2007; Engelkes *et al*. 2008). Therefore, direct interactions between exotic plant species and soil biota, such as release from soil-borne enemies (Reinhart *et al*. 2003), could play an important role in the establishment of exotic plant species. Increased shoot N concentrations of exotics in our study (data not shown) as well as in other studies (e.g. Agrawal *et al*. 2005) may suggest that exotic plant species are able to accelerate nutrient cycling via higher-quality litter inputs (Cornwell *et al*. 2008). Therefore, altered soil nutrient cycling may be a consequence of, rather than a cause for, plant invasions (Ehrenfeld 2010).

In conclusion, our results suggest that exotic plant species do not consistently differ from native congeners in their ability to exploit soil nutrients during their early stage of establishment.

Acknowledgements

We thank Staatsbosbeheer regio Oost for giving us permission to work in Millingerwaard. We thank Wiecher Smant, Maarten Nab, Henk Duyts, Roel Wagenaar, Marco Houtekamer and Ciska Raaijmakers for technical assistance. We thank Mirka Macel, Tim Engelkes, Elly Morriën, Wil Tamis and George Kowalchuk for discussions and advice on the experiment. We thank Roy van Grunsven for help with collecting *Tragopogon dubius* seeds. We thank Jeff Harvey, two anonymous referees and the Handling Editor for valuable suggestions that improved the manuscript. This study was funded by the Dutch Research Council NWO-ALW with a VICI-grant to W.H.v.d.P. This is Publication 5044 of the Netherlands Institute of Ecology (NIOO-KNAW).

References

Aerts, R. & Chapin, F.S. (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research*, **30**, 1–67.

Agrawal, A.A., Kotanen, P.M., Mitchell, C.E., Power, A.G., Godsoe, W. & Klironomos, J. (2005) Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies *Ecology*, **86**, 2979–2989.

Alef, K. & Kleiner, D. (1987) Applicability of arginine ammonification as indicator of microbial activity in different soils. *Biology and Fertility of Soils*, **5**, 148–151.

Bardgett, R.D. (2005) *The Biology of Soil: A Community and Ecosystem Approach*. Oxford University Press, Oxford.

Bardgett, R.D. & Chan, K.F. (1999) Experimental evidence that soil fauna enhance nutrient mineralization and plant nutrient uptake in montane grassland ecosystems. *Soil Biology & Biochemistry*, **31**, 1007–1014.

Bart, J., Fligner, M.A. & Notz, W. (1998) *Sampling and Statistical Methods for Behavioral Ecologists*. Cambridge university press, Cambridge, New York.

Batten, K.M., Scow, K.M., Davies, K.F. & Harrison, S.P. (2006) Two invasive plants alter soil microbial community composition in serpentine grasslands. *Biological Invasions*, **8**, 217–230.

Benjamini, Y. & Hochberg, Y. (1995) Controlling the false discovery rate – a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-Methodological*, **57**, 289–300.

Bever, J.D. (2002) Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **269**, 2595–2601.

Blair, A.C. & Wolfe, L.M. (2004) The evolution of an invasive plant: an experimental study with *Silene latifolia*. *Ecology*, **85**, 3035–3042.

Bodelier, P.L.E., Duyts, H., Blom, C. & Laanbroek, H.J. (1998) Interactions between nitrifying and denitrifying bacteria in gnotobiotic microcosms planted with the emergent macrophyte *Glyceria maxima*. *Fems Microbiology Ecology*, **25**, 63–78.

Bonkowski, M. (2004) Protozoa and plant growth: the microbial loop in soil revisited. *New Phytologist*, **162**, 617–631.

Bonkowski, M., Villenave, C. & Griffiths, B. (2009) Rhizosphere fauna: the functional and structural diversity of intimate interactions of soil fauna with plant roots. *Plant and Soil*, **321**, 213–233.

Boschker, H.T.S. (2004) Linking microbial community structure and functioning: stable isotope (^{13}C) labeling in combination with PLFA analysis. *Molecular Microbial Ecology Manual* (eds G.A. Kowalchuk, F.J. De Bruijn, I.M. Head, A.D. Akkermans & J.D. van Elsas), pp. 3575–3590. Kluwer academic publishers, Dordrecht, The Netherlands.

Chapin, F.S.I., Matson, P.A. & Mooney, H.A. (2002) *Principles of Terrestrial Ecosystem Ecology*. Springer, New York.

Chapuis-Lardy, L., Vanderhoeven, S., Dassonville, N., Koutika, L.S. & Meerts, P. (2006) Effect of the exotic invasive plant *Solidago gigantea* on soil phosphorus status. *Biology and Fertility of Soils*, **42**, 481–489.

Clarholm, M. (1985) Interactions of bacteria, protozoa and plants leading to mineralization of soil-nitrogen. *Soil Biology & Biochemistry*, **17**, 181–187.

Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O. *et al.* (2008) Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters*, **11**, 1065–1071.

De Ridder-Duine, A.S., Smant, W., Van der Wal, A., Van Veen, J.A. & De Boer, W. (2006) Evaluation of a simple, non-alkaline extraction protocol to quantify soil ergosterol. *Pedobiologia*, **50**, 293–300.

Dijkstra, F.A., Bader, N.E., Johnson, D.W. & Cheng, W.X. (2009) Does accelerated soil organic matter decomposition in the presence of plants increase plant N availability? *Soil Biology & Biochemistry*, **41**, 1080–1087.

Dirkse, G.M., Hochstenbach, S.M.H. & Reijerse, A.I. (2007) *Flora van Nijmegen en Kleef 1800-2006/Flora von Nimwegen und Kleve 1800-2006*. KNNV, printed at Zevendal, Mook, The Netherlands.

Duda, J.J., Freeman, D.C., Emlen, J.M., Belnap, J., Kitchen, S.G., Zak, J.C., Sobek, E., Tracy, M. & Montante, J. (2003) Differences in native soil ecology associated with invasion of the exotic annual chenopod, *Halogeton glomeratus*. *Biology and Fertility of Soils*, **38**, 72–77.

Ehrenfeld, J.G. (2003) Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems*, **6**, 503–523.

Ehrenfeld, J.G. (2010) Ecosystem consequences of biological invasions. *Annual Review of Ecology, Evolution and Systematics*, **41**, 59–80.

Engelkes, T., Morriën, E., Verhoeven, K.J.F., Bezemer, M.T., Biere, A., Harvey, J.A., McIntyre, L.M., Tamis, W.L.M. & Van der Putten, W.H. (2008) Successful range expanding plants have less aboveground and belowground enemy impact. *Nature*, **456**, 946–948.

Federle, T.W. (1986) Microbial distributions in soil – new techniques. *Perspectives in Microbial Ecology: Proceedings of the Fourth International Symposium on Microbial Ecology* (eds F. Megusar & M. Gantar), pp. 493–498, Slovene Society for Microbiology, Ljubljana.

Feng, Y.L., Lei, Y.B., Wang, R.F., Callaway, R.M., Valiente-Banuet, A., Inderjit, Li, Y.P. & Zheng, Y.L. (2009) Evolutionary tradeoffs for nitrogen allocation to photosynthesis versus cell walls in an invasive plant. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 1853–1856.

Frostegård, A. & Bååth, E. (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils*, **22**, 59–65.

Frostegård, A., Tunlid, A. & Bååth, E. (1993) Phospholipid fatty-acid composition, biomass, and activity of microbial communities from 2 soil types experimentally exposed to different heavy-metals. *Applied and Environmental Microbiology*, **59**, 3605–3617.

Funk, J.L. & Vitousek, P.M. (2007) Resource-use efficiency and plant invasion in low-resource systems. *Nature*, **446**, 1079–1081.

Harvey, P.H. & Purvis, A. (1991) Comparative methods for explaining adaptations. *Nature*, **351**, 619–624.

Hedlund, K. (2002) Soil microbial community structure in relation to vegetation management on former agricultural land. *Soil Biology & Biochemistry*, **34**, 1299–1307.

Hobbie, S.E. (1992) Effects of plant-species on nutrient cycling. *Trends in Ecology & Evolution*, **7**, 336–339.

Hodkinson, D.J. & Thompson, K. (1997) Plant dispersal: the role of man. *Journal of Applied Ecology*, **34**, 1484–1496.

Ingham, R.E., Trofymow, J.A., Ingham, E.R. & Coleman, D.C. (1985) Interactions of bacteria, fungi, and their nematode grazers – effects on nutrient cycling and plant-growth. *Ecological Monographs*, **55**, 119–140.

Johnson, N.C., Graham, J.H. & Smith, F.A. (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist*, **135**, 575–586.

Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, **17**, 164–170.

Kiers, E.T. & van der Heijden, M.G.A. (2006) Mutualistic stability in the arbuscular mycorrhizal symbiosis: exploring hypotheses of evolutionary cooperation. *Ecology*, **87**, 1627–1636.

Klironomos, J.N. (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, **417**, 67–70.

Klironomos, J.N. (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology*, **84**, 2292–2301.

Koerselman, W. & Meuleman, A.F.M. (1996) The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology*, **33**, 1441–1450.

Kourtev, P.S., Ehrenfeld, J.G. & Haggblom, M. (2003) Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. *Soil Biology & Biochemistry*, **35**, 895–905.

Kuzyakov, Y., Friedel, J.K. & Stahr, K. (2000) Review of mechanisms and quantification of priming effects. *Soil Biology & Biochemistry*, **32**, 1485–1498.

Liao, C., Peng, R., Luo, Y., Zhou, X., Wu, X., Fang, C., Chen, J. & Li, B. (2008) Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. *New Phytologist*, **177**, 706–714.

Mummey, D.L. & Rillig, M.C. (2006) The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. *Plant and Soil*, **288**, 81–90.

Novozamsky, L., Houba, V.J.G., Temminghof, E. & Van der Lee, J.J. (1984) Determination of 'total N' and 'total P' in a single soil digest. *Netherlands Journal of Agricultural Science*, **32**, 322–324.

Olsson, P.A. (1999) Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *Fems Microbiology Ecology*, **29**, 303–310.

Olsson, P.A., Larsson, L., Bago, B., Wallander, H. & van Aarle, I.M. (2003) Ergosterol and fatty acids for biomass estimation of mycorrhizal fungi. *New Phytologist*, **159**, 7–10.

Pringle, A., Bever, J.D., Gardes, M., Parrent, J.L., Rillig, M.C. & Klironomos, J.N. (2009) Mycorrhizal Symbioses and Plant Invasions. *Annual Review of Ecology Evolution and Systematics*, **40**, 699–715.

Reinhart, K.O., Packer, A., Van der Putten, W.H. & Clay, K. (2003) Plant-soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. *Ecology Letters*, **6**, 1046–1050.

Richardson, D.M., Pyšek, P., Rejmánek, M., Barbour, M.G., Panetta, F.D. & West, C.J. (2000) Naturalization and invasion of alien plants: concepts and definitions. *Diversity & Distributions*, **6**, 93–107.

Scharfy, D., Güsewell, S., Gessner, M.O. & Venterink, H.O. (2010) Invasion of *Solidago gigantea* in contrasting experimental plant communities: effects on soil microbes, nutrients and plant–soil feedbacks. *Journal of Ecology*, **98**, 1379–1388.

Schinner, F., Ohlinger, R., Kandeler, E. & Margesin, R. (1995) *Methods in Soil Biology*. Springer-Verslag, Berlin Heidelberg.

Seifert, E.K., Bever, J.D. & Maron, J.L. (2009) Evidence for the evolution of reduced mycorrhizal dependence during plant invasion. *Ecology*, **90**, 1055–1062.

Sharma, G.P. & Raghubanshi, A.S. (2009) Lantana invasion alters soil nitrogen pools and processes in the tropical dry deciduous forest of India. *Applied Soil Ecology*, **42**, 134–140.

Sharpley, A.N. & Withers, P.J.A. (1994) The environmentally-sound management of agricultural phosphorus. *Fertilizer Research*, **39**, 133–146.

Smith, S.E. & Read, D.J. (2008) *Mycorrhizal Symbiosis*, 3rd edn. Elsevier, London.

Tamis, W.L.M., Van der Meijden, R., Runhaar, J., Bekker, R.M., Ozinga, W.A., Ode, B. & Hoste, I. (2005) Anex: standaardlijst van de Nederlandse flora 2003. *Gorteria*, Suppl. **6**, 135–229.

Troelstra, S.R., Wagenaar, R. & De Boer, W. (1990) Nitrification in Dutch heathland soils. *Plant and Soil*, **127**, 179–192.

Van der Meijden, R. (2005) *Heukels' flora van Nederland*, 23 edn. Wolters-Noordhoff bv, The Netherlands.

Van Grunsven, R.H.A., Van der Putten, W.H., Bezemer, T.M., Tamis, W.L.M., Berendse, F. & Veenendaal, E.M. (2007) Reduced plant-soil feedback of plant species expanding their range as compared to natives. *Journal of Ecology*, **95**, 1050–1057.

Verhagen, F.J.M., Hageman, P.E.J., Woldendorp, J.W. & Laanbroek, H.J. (1994) Competition for ammonium between nitrifying bacteria and plant-roots in soil in pots - effects of grazing by flagellates and fertilization. *Soil Biology & Biochemistry*, **26**, 89–96.
Verhoeven, K.J.F., Simonsen, K.L. & McIntyre, L. (2005) Implementing false discovery rate control: increasing your power. *Oikos*, **108**, 643–647.
Vitousek, P.M., Dantonio, C.M., Loope, L.L., Rejmanek, M. & Westbrooks, R. (1997) Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology*, **21**, 1–16.
Vogelsang, K.M. & Bever, J.D. (2009) Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. *Ecology*, **90**, 399–407.
Walling, S.Z. & Zabinski, C.A. (2004) Host plant differences in arbuscular mycorrhizae: extra radical hyphae differences between an invasive forb and a native bunchgrass. *Plant and Soil*, **265**, 335–344.
Weeda, E.J., Westra, R., Westra, C.H. & Westra, F. (1991) *Nederlandse oecologische flora wilde planten en hun relaties 4*. KNNV uitgeverij, Utrecht, The Netherlands.
Williamson, M. & Fitter, A. (1996) The varying success of invaders. *Ecology*, **77**, 1661–1666.
Zou, J.W., Rogers, W.E., DeWalt, S.J. & Siemann, E. (2006) The effect of Chinese tallow tree (*Sapium sebiferum*) ecotype on soil-plant system carbon and nitrogen processes. *Oecologia*, **150**, 272–281.

Received 30 June 2010; accepted 24 May 2011
Handling Editor: Peter Alpert

Supporting Information

Additional supporting information may be found in the online version of this article:

Figure S1. Total plant carbon concentration and total plant biomass.

Figure S2. Concentration of potentially available Phosphorus (P_{olsen}).

Figure S3. Microbial biomass indicators.

Table S1. Mixed-model ANOVA with genus as random factor.

Table S2. Nutrient fluxes in soil with and without plant species.

Table S3. Indicators of microbial biomass in soil with and without plant species.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.