Glomus intraradices and Gigaspora margarita arbuscular mycorrhizal associations differentially affect nitrogen and potassium nutrition of Plantago lanceolata in a low fertility dune soil

Stavros D. Veresoglou · Liz J. Shaw · Robin Sen

Abstract Two controlled microcosm experiments aimed at a critical re-assessment of the contributions of divergent arbuscular mycorrhizal (AM) fungi to plant mineral nutrition were established that specifically targeted Plantago lanceolata–Glomus intraradices (B. B/E) and –Gigaspora margarita (BEG 34) symbioses developed in a native, nutrient limited, coastal dune soil. Plant tissue nitrogen (N), phosphorus (P) and potassium (K) status as well as plant growth parameters and levels of mycorrhizal colonization were assessed at harvest. In addition to the general well-established mycorrhizal facilitation of P uptake, the study was able to demonstrate a G. intraradices-specific contribution to improved plant nitrogen and potassium nutrition. In the two respective experiments, G. intraradices-inoculated plants had 27.8% and 40.8% more total N and 55.8% and 23.3% more total K when compared to Gi. margarita inoculated counterparts. Dissimilar overall contribu-

tion of the two isolates to plant nutrition was identified in AM-genus specific differences in plant tissue N:P:K ratios. G. intraradices inoculated and non-mycorrhizal plants generally exhibited N:P:K ratios indicative of P limitation whereas for Gi. margarita mycorrhizal plants, corresponding ratios strongly implied either N or K limitation. The study provides further evidence highlighting AM functional biodiversity in respect to plant nutrient limitation experienced by mycorrhizal P. lanceolata in an ecologically relevant soil system.

Keywords Plant mineral nutrition · N:P:K ratio · Coastal dune soil · Glomeromycota · Plant nutrient limitation

S. D. Veresoglou (✉) · R. Sen
Department of Environmental and Geographical Sciences,
Manchester Metropolitan University,
John Dalton Building, Chester Street,
M15GD Manchester, UK
e-mail: seby31@zeroone.net

L. J. Shaw
Soil Research Centre, Department of Geography and
Environmental Science, University of Reading,
Reading RG6 6DW, UK

Introduction

Arbuscular mycorrhizas are symbiotic associations formed between the roots of plants and asexual arbuscular mycorrhizal (AM) fungi ascribed to the phylum Glomeromycota (Schüβler et al. 2001) that occur in an estimated 80% of terrestrial plants (Wang and Qui 2006). Maintenance of the symbiosis incurs a carbon cost for the plant host that is generally balanced by a range of benefits provided by the AM fungal mycobiont, including enhanced nutrient uptake, protection from pathogens and improved water relationships (Newsham et al. 1995; Helgason and Fitter 2009; Sikes et al. 2010).

The influence of AM symbiosis on plant mineral nutrition continues to represent a major area of research (Smith and Read 2008) as the outcome is crucial in controlling plant fitness and productivity whilst being relatively easily quantifiable through traditional plant tissue nutrient analysis techniques. Nutritional gains for the host plant from the AM symbiosis have focused mainly on immobile nutrients, namely phosphorus (P), zinc (Zn) and copper (Cu) (Smith and Read 2008), where AM fungi permit plant nutrient acquisition from a more extensive soil volume through development of a extraradical fungal mycelial network that significantly extends the nutrient mobilization zone (Jakobsen et al. 1992; Drew et al. 2003). Despite reports that demonstrate facilitation of nitrogen (N) uptake from organic (e.g., Hodge et al. 2001; Leigh et al. 2009) and mineral (e.g.; Tanaka and Yano 2006) soil resources in the presence of AM fungi, significant enhancement of N nutrition in AM plants remains in dispute (e.g., Reynolds et al. 2005; Smith and Read 2008). Similarly, the case for potassium (K) nutrition still remains unclear. Enhanced K assimilation that has been demonstrated in some studies is often linked with low pH. AM fungi appear to positively contribute to plant K nutrition only under acidic conditions (Clark and Zeto 2000) whilst in other reports the positive effect is linked with AM facilitation of P nutrition in P-deficient soils and the form and availability of N as ammonium or nitrate and even possibly soil Na^+ status (Smith and Read 2008). The latter authors have stressed the need for more defined experimentation to resolve this issue.

In the case of AM-enhancement of host plant P nutrition, there is evidence to suggest different genus- and species-specific contributions to mineral P nutrition (Pearson and Jakobsen 1993; Facelli et al. 2010). However, evidence to suggest that similar AM genus- and species-specific differences operate in relation to N and K assimilation is not as abundant and, when present, has been gained in fertilized soil systems (Azcón et al. 2001). Indeed, a significant amount of our knowledge regarding the nutritional gains for the host plant from the AM symbiosis has focused on agricultural plant species (e.g., Galvez et al. 2001; Marschner et al. 2001) that are adapted to high N, P and K availability and where data has originated from experiments that have utilized

fertilized soils or artificial growth substrates (e.g., Azcón et al. 2008; Hodge et al 2001; Marschner et al. 2001). As such there remains an urgent need to extend these investigations to native plant species in non-fertilized soils of un-managed ecosystems.

Therefore, the aim of this study was to examine the impact of two divergent AM fungi, *Glomus intraradices* and *Gigaspora margarita*, on the growth properties and N, P and K status of *Plantago lanceolata*, in a N, P and K-limited, non-fertilized dune soil over two consecutive experiments. *P. lanceolata* was selected for investigation as it represents a very common mycotrophic perennial plant (Sagar and Harper 1964) in unmanaged-N-limited grasslands (Stevens et al. 2004) and occurred naturally at the site were the soil was obtained. Microcosm experiments utilized the widely studied AM fungal isolates *Glomus intraradices* (B.B/E) and *Glomus margarita* (BEG 34).

It was hypothesized that the two divergent AM fungal isolates, in a series of two experiments, would result in differences in the plant N, P and K status of the corresponding AM fungal inoculated plants with consequences for the type of nutrient limitation experienced by the *P. lanceolata* host. We tested this hypothesis under two experimental conditions: firstly in a system where mycorrhizal *P. lanceolata* plants could exploit soil of similar volume to non-mycorrhizal plants to absorb nutrients (Experiment 1), and further in a microcosm system where mycorrhizal hyphae could access soil unavailable to the *P. lanceolata* root system (Experiment 2).

Materials and methods

Soil

The soil used in both experiments was collected from coastal sand dunes at the Ainsdale Nature Reserve (Merseyside, UK; 53°34′N, 3°5′W). The reserve has Special Site of Scientific Interest (SSSI) and Special Area of Conservation (SAC) status as the nutrient-limited dune slack habitat supports a rich diversity of flora and fauna (Smith 2006; JNCC 2007). The soil was sampled at a depth of 5–15 cm from an area supporting indigenous *P. lanceolata* populations and transported to the laboratory in plastic bags. Soil was air dried on the bench for 2 days, sieved through a

2 mm sieve and sterilized in 5 l volumes by exposure to gamma irradiation (50 kGy for 48 h). The soil was classified as a sandy loam (70% sand, 13% silt, 17% clay) with the following properties: pH: 5.76, organic C 2.06%, Olsen P: 4.97 mg/kg, total P: 148 mg/kg, total N: 1011 mg/kg, total K: 329 mg/kg, total Ca: 105 mg/kg, total Mg: 372 mg/kg, exchangeable ammonia: 29.4 mg/kg, exchangeable nitrate: 6.5 mg/kg.

Experiment 1

Pots, (11 cm height, 8 cm diameter) that were pre-sterilized in bleach and filled with 340 g equivalent dry weight (EDW) of gamma-irradiated Ainsdale dune soil, were used in a controlled experiment. To re-establish a prokaryotic microbial population in the irradiated soil, a microbial suspension was prepared by sequential filtration [Whatman #1 (11 μm pore size) and Whatman #5 (2.5 μm pore size) under vacuum] of a soil extract obtained by orbital shaking (150 rpm) a non-sterile soil:sterile dH$_2$O (1:9 w/v) mixture for 30 min. The microbial suspension was used to raise the soil water content (SWC) to 60% water holding capacity (WHC), and soil was incubated at this SWC (maintained gravimetrically with autoclaved distilled water) in the growth chambers under the experimental growth conditions (see below) for 2 weeks to allow equilibration of the reintroduced microbial community. *Plantago lanceolata* seeds (MAS Seed Specialists, Wiltshire, UK) were surface sterilized through soaking in 70% ethanol (2 min) and 5% bleach in 0.5% SDS (5 min). Following five washings in sterile H$_2$O, seeds were left to germinate on tryptone soy agar plates for 7 days and germinated seeds showing no sign of microbial contamination were used in experiments.

Three treatments were established: non-mycorrhizal, *Glomus intraradices* B.B/E (Biorize France), *Gigaspora margarita* BEG 34 (Biorize, France). The mycorrhizal inoculum comprised 1 g chopped 3-month-old strawberry (*Fragaria vesca*) roots grown semi-aseptically with the respective AM species. The inoculum was positioned 1 cm below a single seedling that was transplanted to each pot. Non-mycorrhizal strawberry roots were used for the non-mycorrhizal control treatments. The experiment consisted of 60 pots (20 per treatment) that were harvested sequentially (in quadruplicate) 2, 3, 5, 7 and 10 weeks after

seedling transfer and AM inoculation. The experimental growth conditions in dedicated plant growth chambers (Fitotron SGC097 equipped with CO$_2$ monitoring and control, Weiss-Gallenkamp, Loughborough, UK) were as follows: day/night photoperiod, temperature and relative humidity of 16/8 h, 22/15°C, 53/70%, respectively and mean ambient CO$_2$ levels of approximately 450 ppm. Throughout the whole experiment pots were watered three times a week with autoclaved distilled water on a gravimetric basis to maintain 60% WHC.

At each harvest, plant material was separated into root and shoot fractions. A small proportion of the root (in the first two harvests approximately 40%; in the other three harvests less than 10%) was sampled for mycorrhizal staining while the rest of the root was dried for 3 days at 80°C. Root dry weight was corrected to account for the mass removed for mycorrhizal staining on the basis of the wet weight-to-dry weight ratio (obtained from the proportion of the root that was dried). The shoot fraction was also dried at 80°C. Percentage of mycorrhizal colonization was assessed on stained roots according to the grid-line intersect method (Giovannetti and Mosse 1980). Oven dried plant material from the 10th week harvest was ball-milled and subjected to an acid digestion as described below.

Experiment 2

Small cylindrical microcosms (12 cm height, 9 cm diameter) were each constructed to include a centrally located longitudinal 40 μm stainless steel mesh divider (Fig. 1). Both equivolume compartments, after sterilization in bleach, were filled with gamma irradiated Ainsdale soil totalling 490 g EDW. The mesh barrier was designed to inhibit the growth of roots, but not AM extraradical hyphae, into the non-planted (root-free) compartment. The bacterial suspension was prepared as in Experiment 1 but instead of H$_2$O, saline phosphate buffer pH 7.2 with 0.1% (v/v) Tween 80 was used as a more efficient microbial extractant and dispersant (Khanmar et al. 2004; Macdonald 1986). Microbial equilibration in soils lasted 3 weeks and the experiment included the following treatments: non-mycorrhizal plants and mycorrhizal plants inoculated with *G. intraradices* or *Gi. margarita* (eight replicate microcosms per treatment). Mycorrhizal inoculum consisted of 20–25
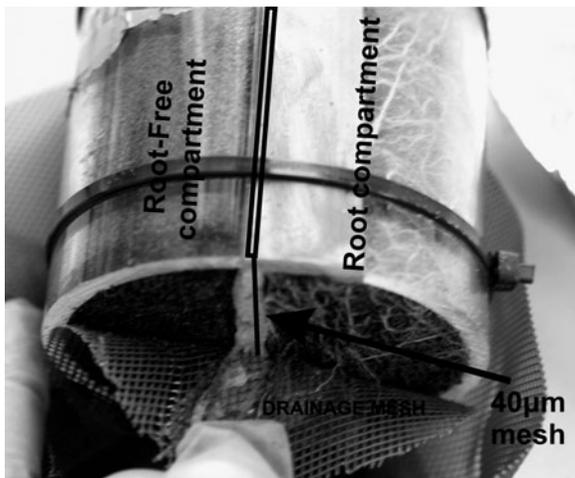
Fig. 1 Photograph of the bottom part of a typical microcosm at harvest in Experiment 2. The 40 µm mesh separates the microcosm into two halves: the rooted compartment were the plant is located, and the root-free compartment. Note the high density of roots in the rooted compartment. The drainage mesh has been partially removed to reveal the texture of the soil

spores, so that the amount of nutrients added in each microcosm would be minimized, that were added to a planting hole approximately 1 cm below the seedling. Spores were isolated from 1-year-old strawberry cultures through the sucrose flotation method (York Mycorrhiza Research Group 2000), surface sterilized for 20 mins in 2% chloramine T supplemented with streptomycin (400 µg/l) according to Giovannetti et al. (1999), and cold shocked overnight at 4°C. The 1-week-old surface sterilized *P. lanceolata* seedlings (prepared as in Experiment 1) were sown so that in each microcosm only one compartment contained a single centrally placed seedling. Plant growth conditions maintained in the Fitotron growth chamber were as in Experiment 1 but in this case CO_2 was actively regulated and maintained in the range 350–380 ppm through computer software control supplemented by a soda lime-based scrubber, which consisted of a tray containing soda lime (~100 g) placed inside the growth chamber that was replaced on a daily basis. Plants were grown for 13 weeks in the growth chamber and, at harvest, treated as in the previous experiment.

Nutrient analysis

Acid digestion was carried out through addition of 2 ml H_2SO_4 and ~100 µg of 1:100 (w / w) selenium:

lithium catalyst to approximately 0.75 g ball-milled plant material. The mix was digested at 360°C for 5 h (Gupta 1987). N (as NH_4^+) and K^+ were determined through ion chromatography (Dionex DX 100 – Dionex Pac CS16 analytical column – IonPacCG16 guard column) while P was assessed through ICP-AES (Varian Vista AX with a CCD detector) analysis at 177.433 nm. Shoots and roots were digested separately and total plant tissue nutrient content was calculated according to the formula:

$$\text{Tissue nutrient content} = [\text{nutrient}]_{shoot} \times \text{mass}_{shoot} \quad (1)$$
$$+ [\text{nutrient}]_{root} \times \text{mass}_{root}$$

Nutrient limitation assessment

To indirectly assess plant growth nutrient limitation type, the approach developed by Hoosbeek et al. (2002) for bog vegetation was generalized. This approach assumes that vegetation N:P and N:K ratios can be indicative of the type of plant growth nutrient limitation experienced. In this respect, a plot is created based on plant sample N:P and N:K ratios and critical N:P, N:K and P:K ratios as established in the literature for the type of vegetation studied.

Data analysis

Statistical analyses were conducted using ANOVA in Minitab version 15 (Minitab, State College, PA). All data were examined for normality using Kolmogorov-Smirnov tests. Multiple comparisons were made according to Fisher's least significant difference (LSD) test (Student 1908), provided that a significant F test at the same significance level was previously obtained. Significance of LSD comparisons of $P \leq 0.05$ was adopted.

Results

Experiment 1

The absence of colonization was verified for all plants assigned to the non-mycorrhizal treatment that did not receive AM inocula, whereas, with the exception of

the first harvest, AM structures were detected in roots of all plants inoculated with *G. intraradices* and *Gi. margarita* (arbuscules + vesicles and arbuscules, respectively). Percent AMF colonization, plant biomass and S:R ratios throughout the experiment are presented in Table 1, and the dry weight status and S:R ratios of the plants in the final harvest are presented in Fig. 2a, b, respectively. There was moderate evidence for a mycorrhizal treatment effect on S:R ratio ($F_{2,27}$=2.54, P=0.097) and plant size ($F_{2,27}$=3.64 P=0.04) across the last three harvests. Data from the first two harvests was ignored as it was not informative due to the small size of the plants.

There was moderate evidence for a treatment effect on *P. lanceolata* N nutrition (P=0.098; Fig. 2c). Shoot tissue P concentrations varied from an average of 0.41 mg/kg (non-mycorrhizal treatment) to 1.22 mg/kg (*Gi. margarita* treatment) (data not shown); values below or close to the minimum shoot tissue P concentrations that have been recorded historically for *P. lanceolata* (0.9 mg/kg) (Güsewell and Koerselman 2002). Mycorrhizal plants were able to forage more efficiently for soil P (Fig. 2d; P<0.05) whilst *G. intraradices* plants had significantly higher K content than those of the two other treatments (P= 0.05) (Fig. 2e). N:P ratio values of total tissue ranged from 17.2 to 94 and N:K ratios ranged from 1.6 to 4 (Fig. 3a). With the exception of *Gi. margarita*-inoculated samples that appeared to be K limited *P.*

lanceolata plants were, according to Hoosberg approach, mainly P limited, (Fig. 3a).

Experiment 2

Both *G. intraradices* and *Gi. margarita* treatments were mycorrhizal at the 13-week harvest, and most plants had flowered or were flowering. Mycorrhizal colonization, assessed according to the grid-line intersect method (mean ± standard error), in these two treatments was 27% (±5.3%) and 26% (±4.4%), respectively. No mycorrhizal structures were detected in non-mycorrhizal plants. Arbuscules in both mycorrhizal treatments were in a state of collapse or had already collapsed, and in the *G. intraradices* treatment mycorrhizal colonization was mainly in the form of vesicles. Colonization of the non-planted root-free compartment was verified through the recovery of spores from this compartment for all the microcosms with mycorrhizal plants. Plant roots had, however, penetrated through the drainage mesh and into the root-free compartment in four, three and six microcosms in non-mycorrhizal, *G. intraradices* and *Gi. margarita* treatments, respectively. Mean dry weights of plants in intact:penetrated microcosms were as follows: 3.3 g:2.3 g for non-mycorrhizal plants, 6.1 g:5.5 g for *G. intraradices* inoculated plants and 4.0 g:5.2 g for *Gi. margarita* inoculated plants. Two sample *t*-tests assuming unequal variances on the impact of root penetration in the root-free

Table 1 Means [± standard error (SE)] of percentage mycorrhizal colonization, shoot:root ratio and plant (shoot + root) dry weight of non-mycorrhizal and mycorrhizal (*Glomus intraradices* or *Gigaspora margarita*) *Plantago lanceolata* plants, over the five sequential harvests that were carried out in the 10-week growth period of Experiment 1 (*n*=4). Shoot:root ratio and plant weight data in the 2nd week of harvest are not presented as the whole root part was used for assessment of mycorrhizal colonization

Treatment	Variable	Time				
		2nd week	3rd week	5th week	7th week	10th week
Non mycorrhizal	Colonization (%)	0%±0%	0%±0%	0%±0%	0%±0%	0%±0%
	Shoot : root		2.08±0.20	1.12±0.19	1.22±0.15	0.89±0.05
	Plant weight (g)		0.02±0.00	0.07±0.02	0.28±0.09	0.68±0.10
G. intraradices	Colonization (%)	3%±1.68%	13%±2.18%	14%±2.79%	30%±3.72%	49%±3.85%
	Shoot : root		2.34±0.49	1.48±0.22	1.91±0.28	0.85±0.02
	Plant weight (g)		0.02±0.00	0.09±0.02	0.40±0.10	1.06±0.27
Gi. margarita	Colonization (%)	11%±2.31%	22%±2.45%	22%±2.13%	62%±2.58%	67%±2.22%
	Shoot : root		2.19±0.29	1.45±0.36	1.580.05	0.97±0.10
	Plant weight (g)		0.02±0.00	0.10±0.04	0.40±0.08	1.34±0.18

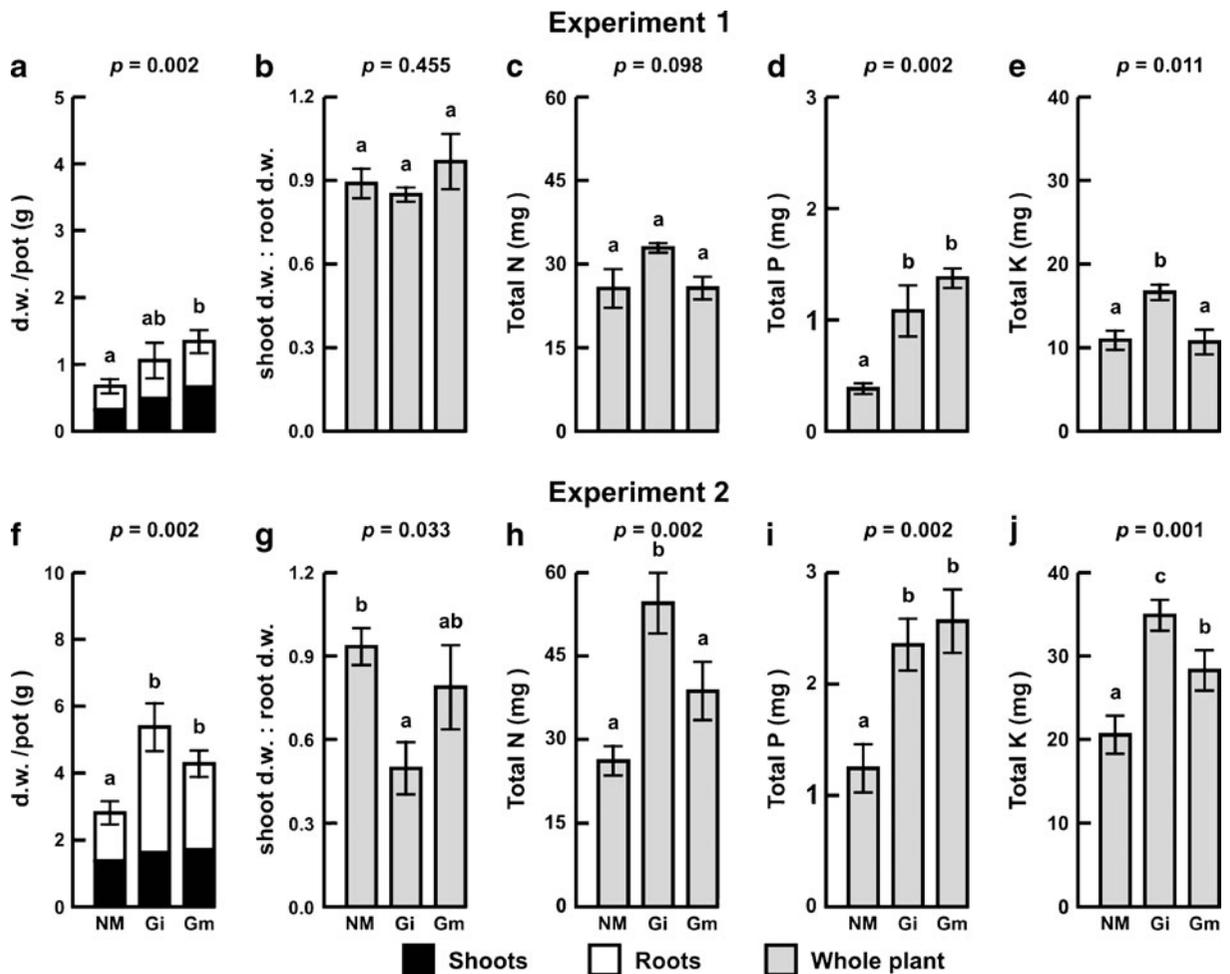## Experiment 1

Experiment 2

Fig. 2a–j Means (± SE) of total dry weight, shoot:root ratio and total tissue N, P and K content in final harvests of Experiments 1 and 2. Total dry weight was calculated as the aggregate of shoot and root dry weight nutrient tissue contents as the aggregate of the shoot and root products of the respective nutrient tissue concentrations with the respective shoot or root dry weights. *P* values above each graph represent the level of significance of the analysis of variance. Treatment means with a different letter are significantly different according to LSD comparisons at *P*=0.05. *NM* Non mycorrhizal, *Gi Glomus intraradices*, *Gm Gigaspora margarita*. **a–e** Experiment 1, **f–j** Experiment 2

compartment on dry weights, N, P and K acquisition revealed that the effect of the rupture on each of the growth parameters was not significant (*P*>0.2). Thus, all samples were included in the analysis as it was speculated that root ingress occurred at a late stage of plant growth and did not greatly affect plant nutrition.

A significant treatment effect was detected with respect to plant biomass as non-mycorrhizal plants were significantly (*P*<0.05) smaller than mycorrhizal plants (Fig. 2f). S:R ratio for *G. intraradices*, was significantly lower than the non-mycorrhizal control (*P*<0.05) (Fig. 2g). N and K nutrition in *G. intraradices*

inoculated plants was significantly (*P*<0.05) enhanced compared to the *Gi. margarita* and non-mycorrhizal plant treatments (Fig. 2h, j) while *Gi. margarita* plants appeared to contain more K than non-mycorrhizal plants (*P*<0.05) (Fig. 2j). There was strong evidence (*P*<0.05) that the P content of mycorrhizal plants was higher (Fig. 2i). N:P ratio of total tissue ranged from 9.88 to 39 and the N:K ratio ranged from 0.6 to 2.3 (Fig. 3b). With the exception of *Gi. margarita*-inoculated samples that experienced all, N-, P- and K- plant-growth nutrient limitation types, the other *P. lanceolata* plants were, according to Hoosberg approach, P limited (Fig. 3b).
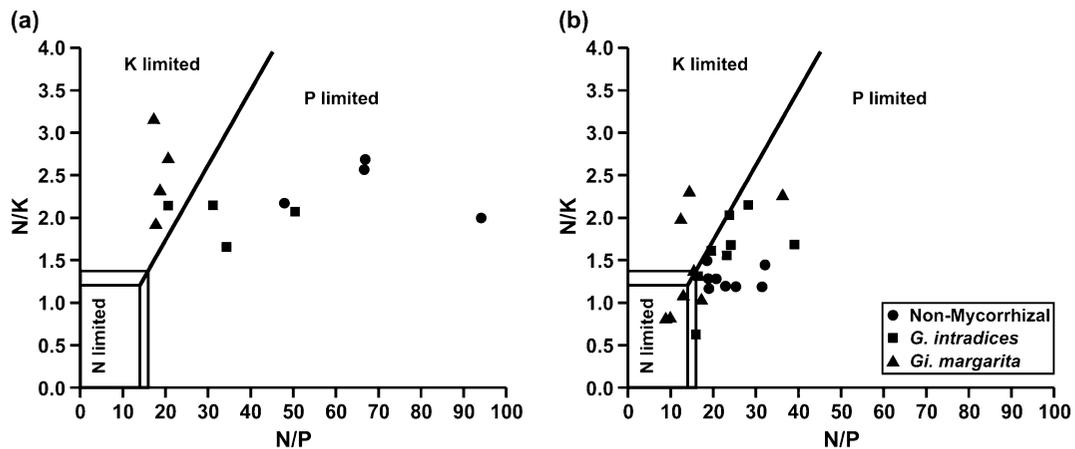
Fig. 3a,b N:P vs N:K ratio plots of individual samples. The nutrient concentrations that were used for the plot of ratios were calculated as total shoot and root nutrient content divided by total plant dry weight. Each plot is comprised by five zones that correspond to N:P:K ratios characteristic of N, P, K limited, N and P co-limited and N and K co-limited plant growth . The critical values approach was adopted from Hoosbeek et al. (2002). Note that the N-P and N-K co-limitation zones are assumed to be narrow. a Experiment 1, b Experiment 2

Discussion

The study presented here examined the effect of AM fungi on the N, P and K status of *P. lanceolata*. In Experiment 1, mycorrhizal and non-mycorrhizal plants had access to nutrients from comparable volumes of soil, whereas in Experiment 2 the microcosm system enabled, probably more realistically, mycorrhizal plants to have access to additional soil volume. This effect combined with the increased duration of the experiment could explain the more pronounced contrasts between non-mycorrhizal controls and mycorrhizal treatments with respect to accumulation of nutrients in mycorrhizal plants in Experiment 2. Elevated CO_2 (ca. 450 ppm) levels in Experiment 1, in addition to the impact on mycorrhizal colonization (Treseder 2004), could have been expected to have permitted enhanced dry weight accumulation per unit of limiting N (Zak et al. 1993), though no such an effect has been detected in the cases when plant growth is limited by P (Edwards et al. 2005). There is no record in the literature, however, to indicate that elevated CO_2 might have any effect on the balance and the proportional plant uptake of nutrients other than N.

Both experiments clearly illustrate the well-known rule that, under P limiting conditions, plant growth is critically dependent on the AM symbiosis in facilitating more efficient P assimilation (Pearson and Jakobsen 1993). More importantly, the data suggest that plants symbiotically associated with *G. intraradices* exhibited more efficient N and K uptake compared to *Gi. margarita* and non-mycorrhizal counterparts. Thus, in agreement with our proposed hypothesis, an AM genus-specific effect was demonstrated for these two nutrients. In fact, comparison between the *Gi. margarita* and non-mycorrhizal treatments in Experiment 1 revealed that, despite the larger size of the mycorrhizal plants, no additional N or K was assimilated in the *Gi. margarita* treatment suggesting not only more efficient K uptake in *G. intraradices* compared to *Gi. margarita* but a *Gi. margarita*-induced repression of N and K uptake when expressed on a plant biomass basis.

The recorded improvement in N and K nutrition could be attributed to either a direct AM fungi effect in enhancing uptake, or, to the better access to soil P that allowed enhanced growth in AM plants, thus increasing N and K uptake as an indirect consequence of the AM symbiosis. *G. intraradices* plants acquired significantly more N (Experiment 2) and K (Experiment 1 and 2). However, in both experiments, *G. intraradices*- and *Gi. margarita*-inoculated plants did not differ significantly in biomass or P uptake, which may suggest that the enhancement in N and K nutrition in the *G. intraradices* treatments, when compared to *Gi. margarita*, was due to a direct influence of *G. intraradices* in nutrient uptake.

Gi. margarita belongs to the family of Gigasporaceae that is distantly related to the Glomeraceae family, which includes *G. intraradices*. In reviewing

studies that have demonstrated facilitation of N uptake by AM fungi, it is worth noting that the majority of these investigated isolates belonged to the family *Glomeaceae*. For example, *Glomus fasciculatum* (Azcón-Aguilar et al. 1993), *Glomus hoi* (Hodge et al. 2001; Leigh et al. 2009), *G. intraradices* (Hawkins et al. 2000; Govindarajulu et al. 2005; Tanaka and Yano 2006; Azcón et al. 2008; Leigh et al. 2009) and *Glomus mosseae* (Ames et al. 1983; Hawkins et al. 2000). Porras-Soriano et al. (2009) investigated inter-species responses within *Glomeaceae* in respect to N, P and K assimilation. In the single study where non-*Glomeaceae* AM fungi were also included, i.e., *Gigaspora gigantea*, *Gigaspora decipiens*, *Archaeospora trappei* (Reynolds et al. 2005), no facilitation in N assimilation was detected although it should be noted that a *Glomus* species that was included in the same experiment, despite having a significant ($P=0.05$) ameliorating effect on total N content compared to the other AM isolates in three out of the five cases examined, did not promote plant N acquisition under the experimental conditions adopted. The results of our study demonstrate differential AM-species-specific N and K assimilation in *P. lanceolata*. Assuming the mechanism to be a result of direct, as opposed to indirect, AM fungal-facilitated uptake, this observation may rest in a distinct physiology of the two families that have so far been demonstrated to differ in morphological and life history traits such as their colonization strategy in terms of respective intraradical and extra-radical allocation of AM fungal biomass (*Gigasporaceae* colonize soil more extensively) and the ability to propagate from root fragments (a low capacity for *Gigasporaceae* isolates) (Hart and Reader 2002; Klironomos and Hart 2002). It is clear that more research is needed to reveal whether, indeed, *Glomus* isolates may possess increased efficiency in facilitating plant N and K nutrition under N and P limiting soil conditions.

To examine if differences in plant nutrient assimilation patterns amongst the treatments may affect the type of plant growth nutrient limitation experienced by *P. lanceolata*, the approach of Hoosbeek et al. (2002) where N:P ratios of samples were plotted against respective N:K ratios was adopted for data derived from Experiment 1 (Fig. 3a) and Experiment 2 (Fig. 3b). Though this approach has been applied for bog vegetation, which has been more extensively studied in terms of growth limitation in the absence

of other limiting factors, an N:P ratio of 15 has consistently been found, irrespective of type of vegetation, to delineate a cut off between P limitation (N:P>15) and N limitation (N:P<15) (Güsewell 2004) with a symmetrical zone of N and P co-limitation for N:P ratios around 15, the extent of which is determined from the characteristics of the ecosystem (Craine et al. 2008). Similarly, there appears to be consistency in the interpretation of N:K ratios (N:K ratios below 1.2 suggest N-limited plant growth and above 1.4, K-limited plant growth) irrespective of type of vegetation (Lawniczak et al. 2009; Venterink et al. 2003). According to Fig. 3a, plant growth in Experiment 1 was limited by either P or K availability, while in Experiment 2 (Fig. 3b), N limitation was also recorded in some samples. However, it was only in the *Gi. margarita* inoculated plants where the N:P:K status shifted from being predominantly P-limited to N- or K-limited. N, P and K limitation are the dominant forms of nutrient limitation in unmanaged terrestrial ecosystems, and are the main controllers of plant growth in the absence of the other growth limiting factors, e.g., light, temperature, diseases and toxicity (Güsewell and Koerselman 2002). That some plant individuals appeared to be N-limited in Experiment 2 may be a result of the longer duration (13 weeks compared to 10 weeks for Experiment 1) of this experiment. Both N and P are mobile nutrients within plants (Taiz and Zeiger 2006). Mature leaves while still photosynthetically active (N demanding process) are no longer growing (P-demanding process) and, therefore, critical N:P values for mature plants appear to be higher (Usuda 1995).

What was interesting in the analytical approach adopted was that the identity of the AM symbiont appeared to affect the type of nutrient limitation that the plant experienced, i.e., P limitation when *P. lanceolata* was inoculated with *G. intraradices* or no AM fungus, and K, P or N when inoculated with *Gi. margarita*. Thus, in agreement with our hypothesis, AM species-mediated differences in *P. lanceolata* N, P and K status manifested in the type of nutrient limitation experienced by the plant host. In the literature, there is evidence that the AM fungi that belong to the family of *Gigasporaceae* (when compared to corresponding AM fungi that belong to *Glomeaceae*) occupy a distinct ecological niche as they are more sensitive to N fertilization (Johnson et al. 2003; Egerton-Warburton et al. 2007), prefer mesic

ecosystems and are unable to cope well with aridity (Egerton-Warburton et al. 2007). As a result, the relative proportion of isolates that belong to the family of *Gigasporaceae* in the AM fungal population may vary considerably across different ecosystems. Working under the assumption that the differences noted in terms of plant nutrition are dependent on the identity of the AM-fungal symbionts, the distinct ecology of the two taxa of AM fungi could become a potentially decisive determinant of the type of growth limitation that the vegetation encounters in natural ecosystems. It should be stressed that the critical N:P: K ratios that were used in this experiment should be treated with caution as they represent indirect indicators of potential nutrient limitations and more targeted experimentation, through addition of nutrients to establish the type of plant growth limitation, is needed to reinforce the inferences made here. However, the possibility that AM fungi can potentially alter the limiting nutrient experienced by their host plant in an AM genus-specific way has implications for the role of AM diversity in influencing the nature of plant–plant interactions in natural ecosystems.

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