Mycorrhiza

Mycorrhizal fungal growth responds to soil characteristics, but not plant host identity, during a primary lacustrine dune succession

--Manuscript Draft--

Manuscript Number:	MCOR-D-13-00074R1
Full Title:	Mycorrhizal fungal growth responds to soil characteristics, but not plant host identity, during a primary lacustrine dune succession
Article Type:	Original Paper
Keywords:	arbuscular mycorrhizas; primary succession; soil development; plant-microbe symbiosis; sand dune
Corresponding Author:	Benjamin Sikes, PhD Kansas Biological Survey and Lawrence, Kansas UNITED STATES
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Kansas Biological Survey and
Corresponding Author's Secondary Institution:	
First Author:	Benjamin Sikes, PhD
First Author Secondary Information:	
Order of Authors:	Benjamin Sikes, PhD
	Hafiz Maherali, PhD
	John Klironomos, PhD
Order of Authors Secondary Information:	
Abstract:	Soil factors and plant host identity can both affect the growth and functioning of mycorrhizal fungi. Both components change during primary succession, but it is unknown if their relative importance to mycorrhizas also changes. This research tested how soil type and plant host differences among primary successional stages determine the growth and plant effects of arbuscular mycorrhizal (AM) fungal communities. Mycorrhizal fungal community, plant identity and soil conditions were manipulated among three stages of a lacustrine sand dune successional series in a fully factorial greenhouse experiment. Late succession AM fungi produced more arbuscules and soil hyphae when grown in late succession soils, although the community was from the same narrow phylogenetic group as those in intermediate succession. AM fungal growth did not differ between host species and plant growth was similarly unaffected by different AM fungal communities. These results indicate that though ecological filtering and/or adaptation of AM fungi occurs during this primary dune succession, it more strongly reflects matching between fungi and soils, rather than interactions between fungi and plant hosts. Thus, AM fungal performance during this succession may not depend directly on the sequence of plant community succession.

Mycorrhizal fungal growth responds to soil characteristics, but not plant host identity, during a primary lacustrine dune succession

Benjamin A. Sikes[1†], Hafiz Maherali[1] and John N. Klironomos[2]

[1]Department of Integrative Biology, University of Guelph, ON, Canada N1G 2W1

[2]Department of Biology, The University of British Columbia – Okanagan, Kelowna, BC, Canada V1V 1V7

† current address: Department of Ecology and Evolutionary Biology and Kansas Biological Survey, University of Kansas, Lawrence, KS 66047, USA

corresponding author: Benjamin A. Sikes

e-mail: ben.sikes@ku.edu

telephone: +785-864-1920

fax: +785-864-1534

Abstract

Soil factors and plant host identity can both affect the growth and functioning of mycorrhizal fungi. Both components change during primary succession, but it is unknown if their relative importance to mycorrhizas also changes. This research tested how soil type and plant host differences among primary successional stages determine the growth and plant effects of arbuscular mycorrhizal (AM) fungal communities. Mycorrhizal fungal community, plant identity and soil conditions were manipulated among three stages of a lacustrine sand dune successional series in a fully factorial greenhouse experiment. Late succession AM fungi produced more arbuscules and soil hyphae when grown in late succession soils, although the community was from the same narrow phylogenetic group as those in intermediate succession. AM fungal growth did not differ between host species and plant growth was similarly unaffected by different AM fungal communities. These results indicate that though ecological filtering and/or adaptation of AM fungi occurs during this primary dune succession, it more strongly reflects matching between fungi and soils, rather than interactions between fungi and plant hosts. Thus, AM fungal performance during this succession may not depend directly on the sequence of plant community succession.

Keywords: arbuscular mycorrhizas; primary succession; soil development; plant-microbe symbiosis; sand dune

Introduction

Soil biota are critical intermediaries in the process of terrestrial succession. For example, microbes in the detrital food web decompose plant material, thus releasing nutrients for themselves and plants (Harte and Kinzig 1993; Wardle et al. 2004). Changes in plant communities alter the quantity and composition of resource substrates within soils (Zak et al. 2003; Waldrop et al. 2006) which in turn alter soil communities by filtering for microbes with specific traits (Waldrop et al. 2004). However, these indirect feedbacks act more slowly than direct feedbacks from soil organisms that parasitize or benefit plant hosts (De Deyn et al. 2003; Kardol et al. 2006). Soil mutualists are thought to be an important source of direct biotic feedback during succession because they help the majority of plants obtain soil resources (Reynolds et al. 2003; Kardol et al. 2006), but little is known about the relative importance of elements that determine mutualist growth, function and potential for feedback during succession.

Arbuscular mycorrhizal (AM) fungi are likely candidates for direct feedbacks because they are the most widespread soil mutualists in nature (Smith and Read 2008) and differences in AM fungal growth traits may alter mycorrhizal function and plant benefits (Powell et al. 2009). AM fungi can facilitate nutrient uptake, increase resistance to water stress, and enhance pathogen protection in exchange for plant photosynthate (Smith and Read 2008; Brundrett 2009). AM fungal traits such as the extent of colonization within roots, in the surrounding soil and the formation of arbuscules for nutrient transfer vary among fungal species with potential functional consequences (Hart and Reader 2002; Powell et al. 2009). For instance, fungal soil hyphae are more effective than plant roots at nutrient uptake from soil because their smaller diameter results in a much larger surface area to volume ratio (Raven and Edwards 2001). As a result, AM fungal species which produce abundant hyphae in soil facilitate enhanced plant nutrient acquisition (Maherali and Klironomos 2007; Powell et al. 2009).

AM fungal growth and the magnitude of the effect that fungi have on their plant hosts can depend on the identity of both the fungus and host, as well as the soil conditions in which the association occurs (Hoeksema et al. 2010; Johnson 2010). AM fungi in the family Gigasporaceae usually concentrate hyphae

in soil while those in the Glomeraceae produce most hyphae within roots (Hart and Reader 2002). Growth and function of AM fungi also differs among plant host species based on traits such as root morphology (Fitter et al. 2004; Sikes et al. 2009). AM fungal growth and function can also be soil specific. Fungi isolated from soils limited in a specific nutrient, such as phosphorus or nitrogen, produce significantly more soil hyphae and arbuscules as well as transfer more limiting nutrients to plant hosts (Johnson et al. 2010) when forming symbioses in these 'home' soils. All three factors: the composition of AM fungal species, plant host identity and soil conditions change over time, therefore succession provides a unique opportunity to test the relative importance of each factor to the growth and functioning of mycorrhizal fungi and their potential to alter plant succession through differential growth benefits to individual plants.

AM fungal species composition, plant host identity and soil conditions were manipulated among three stages of a primary, lacustrine sand dune successional series in a fully factorial greenhouse experiment to determine their relative importance to mycorrhizal fungal growth and plant growth. Previous work from this site demonstrated that AM fungal communities in early succession contain sequences from nearly every AM family in the Glomeromycota. Communities from intermediate and late succession contained sequences only from the genus *Rhizophagus* (Schüßler and Walker 2010) and were dominated by the same single sequence (Sikes et al. 2012). In that study, late successional fungi consistently produced greater numbers of arbuscules and soil hyphae when grown in a common soil combined from all three stages, but no fungal community altered the growth of eight different plant species from across succession (Sikes et al. 2012). Here, it was tested if differences in soil type among successional stages interact with fungal community and host identity to influence fungal traits and benefit to plants. AM fungal communities from each of three stages of succession were grown in each of three successional soil types on one of two plant hosts. Soil types transition from sandy and nutrient poor early successional soils to later successional soils with more organic matter and soil nutrients (Lichter 1998). The two host plants differ in their life history and abundance across succession. *Calamovilfa longifolia* (Hook.) is a rhizomatous grass that dominates in the open dunes from early to intermediate successional stages whereas *Deschampsia flexuosa* (L.) is a major understory bunchgrass within the forest that

dominates from intermediate to late succession (Lichter 1998). We hypothesized that AM fungal growth and benefit to hosts would be determined by interactions among all three elements, but soil type would have the strongest effect based on its importance in filtering AM fungal communities (Ji et al. 2012; Schechter and Bruns 2013; Doubková et al. 2013).

Methods

Field Collection and Preparation

Each successional component including soils, seeds and fungi was collected and isolated from the successional series at Wilderness State Park, Michigan, USA (45°43' N, 84°56' W) as previously described in Sikes et al (2012). The two experiments were setup in parallel and each component was identical. Soils were collected during June 2007 from three pairs of dunes that represent distinctly different stages in both plant community composition and edaphic conditions including soil pH and soil nutrients (Lichter 1998). Our youngest dunes were 10 and 35 years old respectively, intermediate-aged dunes formed 235-295 years ago, and late successional dunes formed 450 and 845 years ago (Lichter 1997). We also collected seeds of *C. longifolia* and *D. flexuosa* from multiple individuals across the successional series throughout the summer of 2007.

AM fungal inoculum from each successional stage was isolated using repeated sucrose-centrifugation on pooled soil cores from dunes of similar ages (Sikes et al. 2012). Pooled soils were combined from 10 random points along paired dunes. Spores and hyphae were cleaned and hydrated in 100 ml of autoclaved, de-ionized water (final concentration 18g soil/ml inoculum). To control for differences in microbial contaminants introduced with each AM fungal community, we also collected a microbial filtrate by passing the initial spore collections through a 25-μm sieve. Microbial filtrates from all dunes were combined to represent a common microbial wash added as a control (Koide and Li 1989). Inoculum from each AM fungal community and microbial wash was maintained at 4°C for two weeks prior to plant inoculation.

Soils were sterilized by gamma-irradiation to 32 kGy (McNamara et al. 2003). Forty sterilized soil cores from paired successional stages (twenty from each) were combined to form each soil type: 'Early', 'Intermediate', or 'Late'. This soil was sieved through a sterilized 6-cm sieve to remove larger organic material that could bias individual pots. In addition, we sterilized standard 'play' sand (Hillview; Ontario, Canada) by autoclaving for one hour. Soils were used to fill mini-tree pots (6.35cm w X 25.4cmh, 857ml vol.; Stuewe and Sons; Oregon, USA) for each experimental unit. Each replicate pot consisted of 300ml of 'play' sand on bottom and 600 ml of gamma-irradiated field soil on top.

Soil Nutrient Analysis

Differences in soil chemistry and nutrients were assessed for three replicate sub-samples of each initial soil type. Each sample was analyzed for soil pH (Hendershot et al. 1993), phosphorus (sodium bicarbonate extraction- (Reid 2006), calcium and magnesium (Ammonium Acetate extraction- (Simard 1993), Total Soil Carbon (Combustion Method- (LECO Corporation 2011), and soil ammonium and nitrate (KCl extraction). All analyses except soil ammonium and nitrate were carried out at University of Guelph, Lab Services. Soil ammonium and nitrate were extracted with KCl and analyzed using spectrophotometry (Maynard and Kalra 1993).

Experimental Setup and Growth

Seeds from each plant species were surface sterilized, stratified and germinated as in Sikes et al (2012). Seedlings were transplanted within three days of germination. Plants were allowed to grow for two weeks and any seedlings that died following transplantation were replaced. After two weeks, each plant species was inoculated with one of the following AM fungal treatments: 1) sterile water control, 2) microbial wash only, 3) microbial wash + AM fungi from early succession dunes, 4) microbial wash + AM fungi from intermediate age dunes, or 5) microbial wash + AM fungi from late succession dunes. One ml of fungal inoculum was added directly to the root area using a sterile pipette inserted slightly below the soil surface. One ml of microbial wash was subsequently added in the same way. Each treatment combination (5 AM fungal additions X 3 soils X 2 plants = 30 in total) was replicated 10 times for a total of 300 experimental units. Pots were arranged in a randomized complete block design. Drip

irrigation was used to provide 5ml of water to the pots three times a day and plants were not fertilized (in contrast to Sikes 2012).

Plants were grown for four months at which time most individuals were still in vegetative growth with a few larger individuals of both species flowering (less than 10% of all individuals). Plants were harvested and aboveground plant biomass was weighed, dried at 55°C for 3 days and then re-weighed. Plant roots were gently shaken free of soil and washed on a 1-mm sieve for up to 15 min to remove soil particles. Roots were then briefly air dried, weighed, then sub-sampled to stain for arbuscular mycorrhizal structures. The mass of roots sub-sampled for staining varied with the total root biomass available from harvested plants. The average amount taken was ~320mg of wet root material up to a maximum of 700mg. Plants that were either dead or had very low root biomass (<100mg) were not sampled so as to reduce error propagation in biomass measurements. The same quantity of stained roots was used for all fungal quantification (see below). The remaining root biomass was dried as above, then reweighed and final root dry weight estimated by simple proportion (total root wet / total root dry = post-sample root wet/ post-sample root dry). Soils from each replicate were homogenized and 100mg was taken for quantification of extra-radical soil fungal hyphae.

Differential staining and microscopy was used to examine differences in AM fungal growth characteristics. AM fungal structures and colonization were quantified using the magnified intersect method (McGonigle et al. 1990) after roots were stained with Chlorazol Black E (Brundrett et al. 1994). AM fungal hyphae were distinguished from other hyphae based on the presence of coenocytic hyphae. Eighteen (2-cm long) root fragments were randomly selected from each subsample and mounted onto two glass slides. For each experimental unit, the presence of arbuscules (the site of exchange between plant and fungus), vesicles (storage structures) and intra-radical hyphae were assessed at 150 intersections. Soil hyphal length was determined by dissolving soil aggregates with sodium hexametaphosphate and then staining and visualizing as above. Hyphal intersections were then converted to hyphal length (Hart and Reader 2002).

Statistical Analysis

Initial soil characteristics were analyzed using analysis of variance (ANOVA) for each edaphic variable (soil pH and individual soil nutrients) with soil successional stage (early, intermediate, or late) as the independent variable. It was tested if fungal growth traits, both within and outside plant roots were affected by AM fungal successional stage, soil type, plant identity or their interactions. ANOVAs were run with either number of arbuscules, vesicles, intra-radical hyphae, or soil hyphae as dependent variables and AM fungal successional stage, soil type and plant host species as independent variables and with block as a random factor. 'Control' and 'wash' treatments were excluded from analyses with arbuscules and vesicles because these structures were completely absent from those treatments. Replicates where no roots were taken due to lack of material were also excluded.

The effects of the successional origin of AM fungi, soil type or plant host on total plant biomass, root biomass, shoot biomass, and the ratio of root biomass to total biomass were determined using multi-factor ANOVA models as above.

For all analyses, Tukey post-hoc tests were used on significant factors to analyze specific pair-wise comparisons. All analyses were conducted in R (R Development Core Team 2011) and graphics were created in SigmaPlot 11.0 (Systat Software; San Jose, CA) .

Results

Initial Differences among Soil Types

Successional soil types differed in soil pH and all measured soil nutrients (Figure 1, pH: $F_{2,6}$= 5558, $p<0.0001$; P: $F_{2,6}$=337.28, $p<0.0001$; Mg: $F_{2,6}$= 76.121, $p<0.0001$; K: $F_{2,6}$= 670.88, $p<0.0001$, C: $F_{2,6}$= 221.09, $p<0.0001$; NO_3: $F_{2,6}$= 1850.4, $p<0.0001$; NH_4: $F_{2,6}$= 71.85, $p<0.0001$, Supp. Material). Early successional soils were the most basic ($p<0.0001$ for all pairwise comparisons) and contained more nitrate than other soils ($p<0.0001$). Intermediate successional soils had the most total magnesium ($p<0.0001$) but values for all other edaphic factors fell in-between early and late successional soils. Late successional soils were the most acidic and had the most total phosphorus, carbon, potassium, and ammonium ($p<0.0001$, Figure 1).

Differences in Mycorrhizal Traits

AM fungal traits were influenced by an interaction between AM fungal successional stage and soil type, but not plant species. This interaction was driven by late successional AM fungi, which produced more arbuscules than either early or intermediate AM fungi when in late successional soil, but significantly less arbuscules than other fungi when in early successional soil ($F_{4,127}$= 29.20, p<0.0001, Figure 2A, ANOVA tables in Supp. Material). Late successional AM fungi also produced significantly more soil hyphae than other AM fungi when placed in late successional soil ($F_{8,253}$= 4.41, p<0.0001, Figure 2B). There was a statistically significant difference in intra-radical hyphae among sources of fungal inocula ($F_{4,221}$= 2.81, p<0.05) but there were no significant pairwise differences (p>0.05 for all pairwise comparisons). Hyphae in 'control' and 'wash' treatments were likely a product of background colonization by non-AM fungi. The density of vesicles did not differ among any factors.

Plant Responses to AM fungi and soils

The successional stage of AM fungi did not influence the biomass of plant hosts. Regardless of the particular measure of biomass (total, shoot, or root), plant growth did not differ among AM fungal inocula (total biomass: $F_{4,253}$= 1.91, p=0.109, Figure 3, Supp. Material). There were also no significant interactions among AM fungal successional stage, soil type and plant species. Both plant species did have higher growth in intermediate and late successional soils than in early successional soil (total biomass: $F_{2,253}$= 1096.19, p<0.0001). *D. flexuosa* biomass increased by 2139% on average from early to intermediate successional soils, whereas *C. longifolia* biomass increased by 985% on average. Both plant species allocated more biomass to roots in early successional soils than those in intermediate and late successional soils ($F_{2,253}$= 75.73, p<0.0001, Supp. Material), with *D. flexuosa* shifting more biomass to shoots than *C. longifolia* in intermediate and late succession soils (soil X plant $F_{2,253}$= 7.62, p<0.001; Figure 4).

Discussion

These results indicate that AM fungal growth within this dune succession depends more on the successional stage of the fungal community and the specific soil environment than the host plant (Schechter and Bruns 2013). Late successional AM fungi drove this pattern producing the most arbuscules and soil hyphae when forming symbioses in their 'home' soil, and producing the fewest arbuscules in early successional soil. Early and intermediate successional AM fungi did not differ in growth among soil types. The two plant hosts had distinct growth forms, and host growth responded to soil type, but host differences in growth and biomass allocation did not influence the growth of AM fungi from any successional stage. The rapid soil development in this dune succession (Lichter 1998) resulted in substantial differences in edaphic properties among the successional stages represented in our experiment, and these differences may overwhelm any smaller host –derived differences to AM fungal growth. These results indicate that these abiotic changes were more important in determining AM fungal growth than biotic interactions that could have differed between these specific hosts species (Bever 2002; Kiers et al. 2011).

Differences in the formation of arbuscules by late succession AM fungi may reflect a fitness trade-off (Kawecki and Ebert 2004), as a result of late succession AM fungal adaptation to specific soil characteristics. The relatively uniform traits and performance of early and intermediate successional AM fungi across the soil environments suggests that adaptation did not occur in fungi from these environments. However, because we evaluated fungal traits and performance on entire communities of AM fungi, we cannot unambiguously attribute the patterns we observed to adaptation as they may also be a product of differences in phenotypes or communities. Future tests of AM adaptation as a mechanism explaining differences in fungal traits and performance in contrasting soil environments should be done using reciprocal transplants with individual species (e.g., Sherrard and Maherali 2012).

Ecological filtering of AM fungal communities and phenotypic responses of fungi to physical and chemical differences in the soil types could also be mechanisms for differences in AM fungal growth. Species in the diverse early successional AM fungal community are absent from intermediate and late succession, indicating that AM community filtering occurs during succession (Sikes et al. 2012).

Intermediate and late succession AM fungi consisted of sequences solely from *Rhizophagus*, including one OTU that accounted for 70% of all sequences detected in both stages (Sikes et al. 2012). AM fungal diversity was not assessed at the end of the experiment to determine if community changes during the experiment could have resulted in fungal trait differences among soil types. Community divergence between intermediate and late succession AM fungi could have produced distinct growth responses, but early and intermediate succession AM fungal communities could not have converged because they did not overlap in species. The differences in trait responses to soil type between nearly identical starting AM fungal communities may further indicate the importance of examining variation below the species level for understanding mycorrhizal responses to the environment (Koch et al. 2006). In our study, we could not determine whether specific fungal genotypes, species or genera responded to differences in soil type. Given this uncertainty, future work should determine the degree to which variation at each of these levels affects mycorrhizal traits and performance.

Differences in fungal growth did not predict their effects on plant growth, possibly as a result of strong differences in soil nutrient limitation. AM soil hyphae facilitate soil resource acquisition and arbuscules facilitate the transfer of these nutrients to plant roots (Smith and Read 2008; Powell et al. 2009). Yet the soil-specific variation in arbuscules and hyphal growth of late successional AM fungi was not associated with any differences in plant biomass. The lack of an association between plant growth and increased soil hyphae and arbuscles may have been caused by variation in soil nutrient levels across soil types. Phosphorus was higher in the late successional soils compared to previous field observations (Lichter 1998), despite careful collection and storage of soils as well as soil sterilization that should minimize nutrient flushes (McNamara et al. 2003). Bicarbonate extraction also likely underestimated phosphorus in the acidic late successional soils (Olsen et al. 1954). Increased phosphorus may have negated any plant growth benefit from the increased hyphae and arbuscules of late succession AM fungi (Collins and Foster 2009). Mycorrhizal effects on hosts may have also been reduced because plants in the greenhouse were limited by soil resources that AM fungi were unable to provide in sufficient quantity. For example, N:P ratios were low in intermediate (N:P = 5.1) and late successional (N:P = 4.0) soils

(Johnson 2010), indicating that plant growth may have been limited by N and not P. If no AM fungi could provide N to plants, then plant growth would have been unaffected by fungal presence. Nevertheless, the lack of fungal growth effects on plants suggests that mycorrhizal fungi can respond directly to soils independently of their influence on plant host growth.

Soil pathogens may also be a missing component necessary to understand the lack of mutualist benefits among successional stages. The "wash" treatment has previously been used as a saprophyte/pathogen treatment to quantify negative interactions (Klironomos 2002). Only *C. longifolia* in late succession soils showed reduced growth with this treatment compared to control plants. In this study, the "wash" microbial fractions from each successional stage were combined and this pooling could have eliminated stage specific interactions between AM fungi, pathogens/saprobes and hosts. Given the specificity of these interactions (Borowicz 2001; Sikes et al. 2009), it is likely that only certain combinations within a specific soil may have resulted in mycorrhizal benefits. The relative importance of negative and positive soil biotic feedbacks may differ among dune successional stages as it does in secondary succession (Kardol et al. 2006). Kardol et al. (2006) found that negative soil feedbacks were stronger in early succession while positive feedbacks, attributed to mycorrhizal fungi, were stronger in late succession. A combined "wash" inoculum may have reduced the dominance of stage-specific pathogens and thereby eliminated any potential mycorrhizal benefits from pathogen protection.

Our results indicate that soil context is an essential determinant of the growth of a widespread soil mutualist across a successional sequence. The lack of effects by either host suggests obligate mutualists can respond to abiotic environments with little regard for (or effect on) their host. If this type of host independent response to soil environment is common, then there are implications not only for predicting how ecological conditions affect the symbiosis (Johnson 2010; Doubková et al. 2013), but also for understanding how and why it remains stable over evolutionary time (Thrall et al. 2007). For example, if fungal adaptation to soil environments can occur while having neutral effects on hosts, natural selection on AM fungi by the physical environment may be stronger than natural selection imposed by fungal hosts. Our finding that AM fungal traits and performance were decoupled from effects on plant hosts

suggests that soil nutrient conditions can influence AM fungi in ways that are not predictable from plant resource limitation alone (Johnson 2010). Therefore, explaining how soil mutualists affect plant succession requires the explicit incorporation of changes in soil development as a mechanism independent from plant host identity and host resource requirements.

Acknowledgements:

The authors thank Kevin Courtney, Michelle Doucette, Lindsay Wilson and Michael Mucci for help with maintaining the experiment and data collection. BAS was partially supported by an Arthur Richmond Scholarship and an International Scholarship from the University of Guelph. HM and JNK wish to thank the Natural Sciences and Engineering Research Council of Canada (NSERC) for funding.

References:

Bever J (2002) Host-specificity of AM fungal population growth rates can generate feedback on plant growth. Plant and Soil 244:281–290.

Borowicz VA (2001) Do arbuscular mycorrhizal fungi alter plant–pathogen relations? Ecology 82:3057–3068.

Brundrett M (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant and Soil 320:37–77. doi: 10.1007/s11104-008-9877-9.

Brundrett M, Melville L, Peterson L (1994) Practical methods in mycorrhiza research. Mycologue Publications, Waterloo, Canada.

Collins CD, Foster BL (2009) Community-level consequences of mycorrhizae depend on phosphorus availability. Ecology 90:2567–2576.

De Deyn GB, Raaijmakers CE, Zoomer HR, et al. (2003) Soil invertebrate fauna enhances grassland succession and diversity. Nature 422:711–713.

Doubková P, Kohout P, Sudová R (2013) Soil nutritional status, not inoculum identity, primarily determines the effect of arbuscular mycorrhizal fungi on the growth of *Knautia arvensis* plants. Mycorrhiza. doi: 10.1007/s00572-013-0494-y.

Fitter AH, Heinemeyer A, Husband R, et al. (2004) Global environmental change and the biology of arbuscular mycorrhizas: gaps and challenges. Canadian Journal of Botany 82:1133–1139. doi: 10.1139/b04-045.

Hart MM, Reader RJ (2002) Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytologist 153:335–344.

Harte J, Kinzig AP (1993) Mutualism and competition between plants and decomposers: implications for nutrient allocation in ecosystems. The American Naturalist 141:829–846.

Hendershot WH, Lalande H, Duquette M (1993) Soil reaction and exchangeable acidity. In: Carter MR (ed) Soil sampling and methods of analysis. CRC Press, Boca Raton,FL, pp 141–146.

Hoeksema JD, Chaudhary VB, Gehring CA, et al. (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecology Letters 13:394–407.

Ji B, Bentivenga SP, Casper BB (2012) Comparisons of AM fungal spore communities with the same hosts but different soil chemistries over local and geographic scales. Oecologia 168:187–197.

Johnson NC (2010) Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. New Phytologist 185:631–647. doi: 10.1111/j.1469-8137.2009.03110.x

Johnson NC, Wilson GWT, Bowker MA, et al. (2010) Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proceedings of the National Academy of Sciences 107:2093–2098.

369 Kardol P, Martijn Bezemer T, van der Putten WH (2006) Temporal variation in plant-soil feedback
370 controls succession. Ecology Letters 9:1080–1088.

371 Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecology Letters 7:1225–1241.

372 Kiers ET, Duhamel M, Beesetty Y, et al. (2011) Reciprocal Rewards Stabilize Cooperation in the
373 Mycorrhizal Symbiosis. Science 333:880–882. doi: 10.1126/science.1208473

374 Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in
375 communities. Nature 417:67–70.

376 Koch AM, Croll D, Sanders IR (2006) Genetic variability in a population of arbuscular mycorrhizal fungi
377 causes variation in plant growth. Ecology Letters 9:103–110.

378 Koide RT, Li M (1989) Appropriate controls for vesicular-arbuscular mycorrhiza research. New
379 Phytologist 111:35–44.

380 LECO Corporation (2011) LECO SC-444 Instruction Manual, Michigan, USA.

381 Lichter J (1998) Primary Succession and Forest Development on Coastal Lake Michigan Sand Dunes.
382 Ecological Monographs 68:487–510.

383 Lichter J (1997) AMS radiocarbon dating of Lake Michigan beach-ridge and dune development.
384 Quaternary Research 48:137–140.

385 Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly and
386 ecosystem functioning. Science 316:1746–1748. doi: 10.1126/science.1143082

387 Maynard DG, Kalra YP (1993) Nitrate and exchangeable ammonium nitrogen. Soil sampling and
388 methods of analysis. CRC Press, Boca Raton,FL, pp 25–38

389 McGonigle TP, Miller MH, Evans DG, et al. (1990) A new method which gives an objective measure of
390 colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 115:495–501.

391 McNamara NP, Black HIJ, Beresford NA, Parekh NR (2003) Effects of acute gamma irradiation on
392 chemical, physical and biological properties of soils. Applied Soil Ecology 24:117–132.

393 Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus by extraction
394 with sodium bicarbonate (Circular 39). USDA, Washington, DC.

395 Powell JR, Parrent JL, Hart MM, et al. (2009) Phylogenetic trait conservatism and the evolution of
396 functional trade-offs in arbuscular mycorrhizal fungi. Proceedings of the Royal Society B
397 276:4237–4235.

398 R Development Core Team (2011) R: A Language and Environment for Statistical Computing. Vienna,
399 Austria.

400 Raven JA, Edwards D (2001) Roots: evolutionary origins and biogeochemical significance. J Exp Bot
401 52:381–401.

402 Reid K (2006) Soil fertility handbook. Ministry of Agriculture, Food and Rural Affairs, Ontario, Canada.

403 Reynolds HL, Packer A, Bever JD, Clay K (2003) Grassroots ecology: plant-microbe-soil interactions as
404 drivers of plant community structure and dynamics. Ecology 84:2281–2291.

405 Schechter SP, Bruns TD (2013) A common garden test of host-symbiont specificity supports a dominant
406 role for soil type in determining AMF assemblage structure in Collinsia sparsiflora. PLoS ONE
407 8:e55507.

408 Schüßler A, Walker C (2010) The Glomeromycota: a species list with new families and new genera. The
409 Royal Botanic Garden Kew, Botanische Staatssammlung Munich, and Oregon State University.
410 http://schuessler.userweb.mwn.de/amphylo/Schuessler&Walker2010_Glomeromycota.pdf
411 Accessed 18 September 2013.

412 Sherrard ME, Maherali H (2012) Local adaptation across a fertility gradient is influenced by soil biota in
413 the invasive grass, *Bromus inermis*. Evol Ecol 26:529–544. doi: 10.1007/s10682-011-9518-2.

414 Sikes BA, Cottenie K, Klironomos JN (2009) Plant and fungal identity determines pathogen protection of
415 plant roots by arbuscular mycorrhizas. Journal of Ecology 97:1274–1280.

416 Sikes BA, Maherali H, Klironomos JN (2012) Arbuscular mycorrhizal fungal communities change among
417 three stages of primary sand dune succession but do not alter plant growth. Oikos 121:1791–
418 1800. doi: 10.1111/j.1600-0706.2012.20160.x.

419 Simard RR (1993) Ammonium acetate-extractable elements. In: Carter MR (ed) Soil sampling and
420 methods of analysis. Lewis Publishers, Boca Raton,FL, pp 39–42.

421 Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic press, San Diego.

422 Thrall PH, Hochberg ME, Burdon JJ, Bever JD (2007) Coevolution of symbiotic mutualists and parasites
423 in a community context. Trends in Ecology & Evolution 22:120–126.

424 Waldrop MP, Zak DR, Blackwood CB, et al. (2006) Resource availability controls fungal diversity across
425 a plant diversity gradient. Ecology Letters 9:1127–1135.

426 Waldrop MP, Zak DR, Sinsabaugh RL (2004) Microbial community response to nitrogen deposition in
427 northern forest ecosystems. Soil Biol Biochem 36:1443–1451. doi:
428 10.1016/j.soilbio.2004.04.023.

429 Wardle DA, Bardgett RD, Klironomos JN, et al. (2004) Ecological linkages between aboveground and
430 belowground biota. Science 304:1629–1633.

431 Zak DR, Holmes WE, White DC, et al. (2003) Plant diversity, soil microbial communities, and ecosystem
432 function: Are there any links? Ecology 84:2042–2050. doi: 10.1890/02-0433.

433

434

435

436

Figure 1 Total soil phosphorus (A), carbon (B), potassium (C), magnesium (D), nitrate (E), ammonium (F), and soil pH (G) among soil types. Error bars represent standard error of the mean. Letters indicate significant pairwise differences ($p < 0.05$) in each factor among soil types. Soil types are pooled from respective successional stage as outlined in *Methods*: Early-soils from dunes 15-35 years old, Intermediate (Inter.)-soils from dunes 235-295 years old, Late-soils from dunes 450-835 years old

Figure 2: Arbuscule (% root length colonized) and soil hyphal length (cm) for individual treatments based on AM fungal inoculum, soil type and plant species. AM fungal additions are as follows 'Control'- water only, 'Wash'- microbial wash only, 'Early'- microbial wash + early successional AM fungi, 'Intermediate'- microbial wash + intermediate successional AM fungi, and 'Late'- microbial wash + late successional AM fungi. Legend indicates colors for each fungal inoculum added. Letters below the figure indicate significant pairwise differences ($p < 0.05$) in combinations of AM fungal additions and soil type. There were no arbuscules in either control or wash treatments therefore they were excluded from the graphs. Soil types and symbols are as in Figure 1. Plant species are C. longifolia- *Calamovilfa longifolia* (early successional) and D. flexuosa- *Deschampsia flexuosa* (late succession).

Figure 3: The effect of soil type and inoculum addition on total biomass for each plant species. All abbreviations and inocula are as in figure 2. Letters below the figure indicate significant pairwise differences ($p < 0.05$) between soil types.

Figure 4: The ratio of root to total biomass for each plant species grown in each soil type and with each AM fungal inoculum. All abbreviations and symbols are as in figure 2.

Fig1

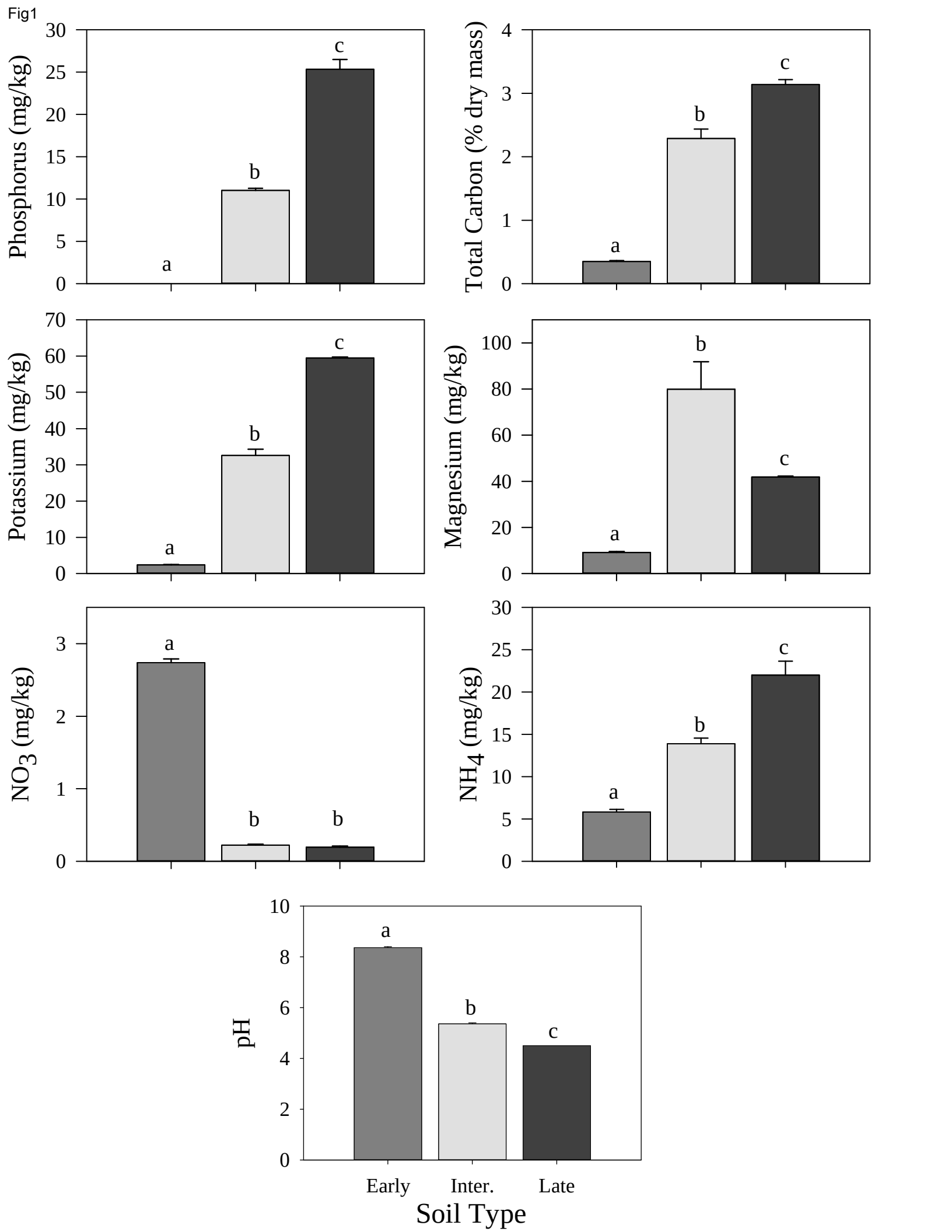
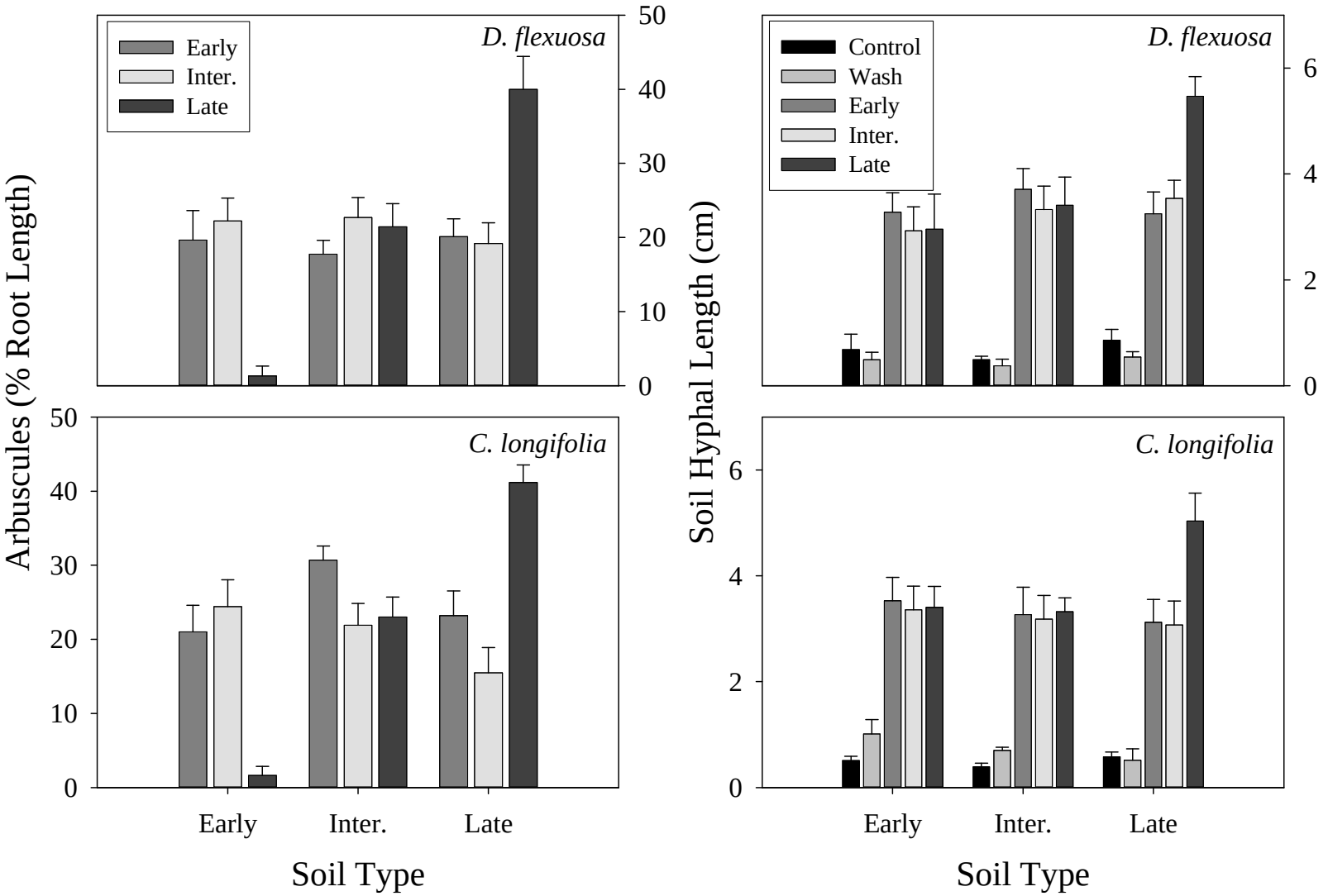

Phosphorus (mg/kg)
Total Carbon (% dry mass)
Potassium (mg/kg)
Magnesium (mg/kg)
NO3 (mg/kg)
NH4 (mg/kg)
pH
a
b
c
Early
Inter.
Late
Soil Type

Fig2



Soil:Microbe b b a b b b b b c

a a b b b a a b b b a a b b c

Fig3

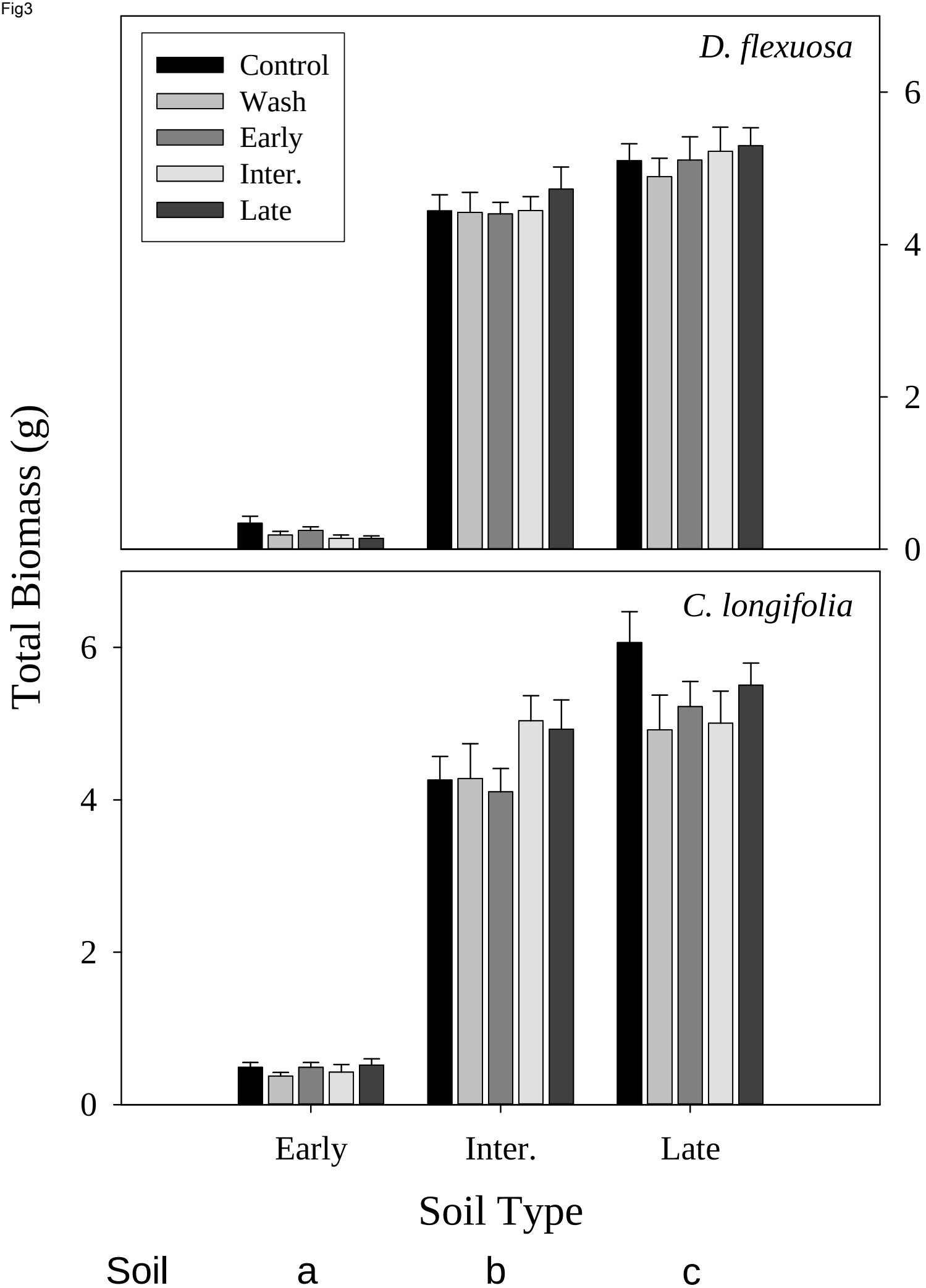
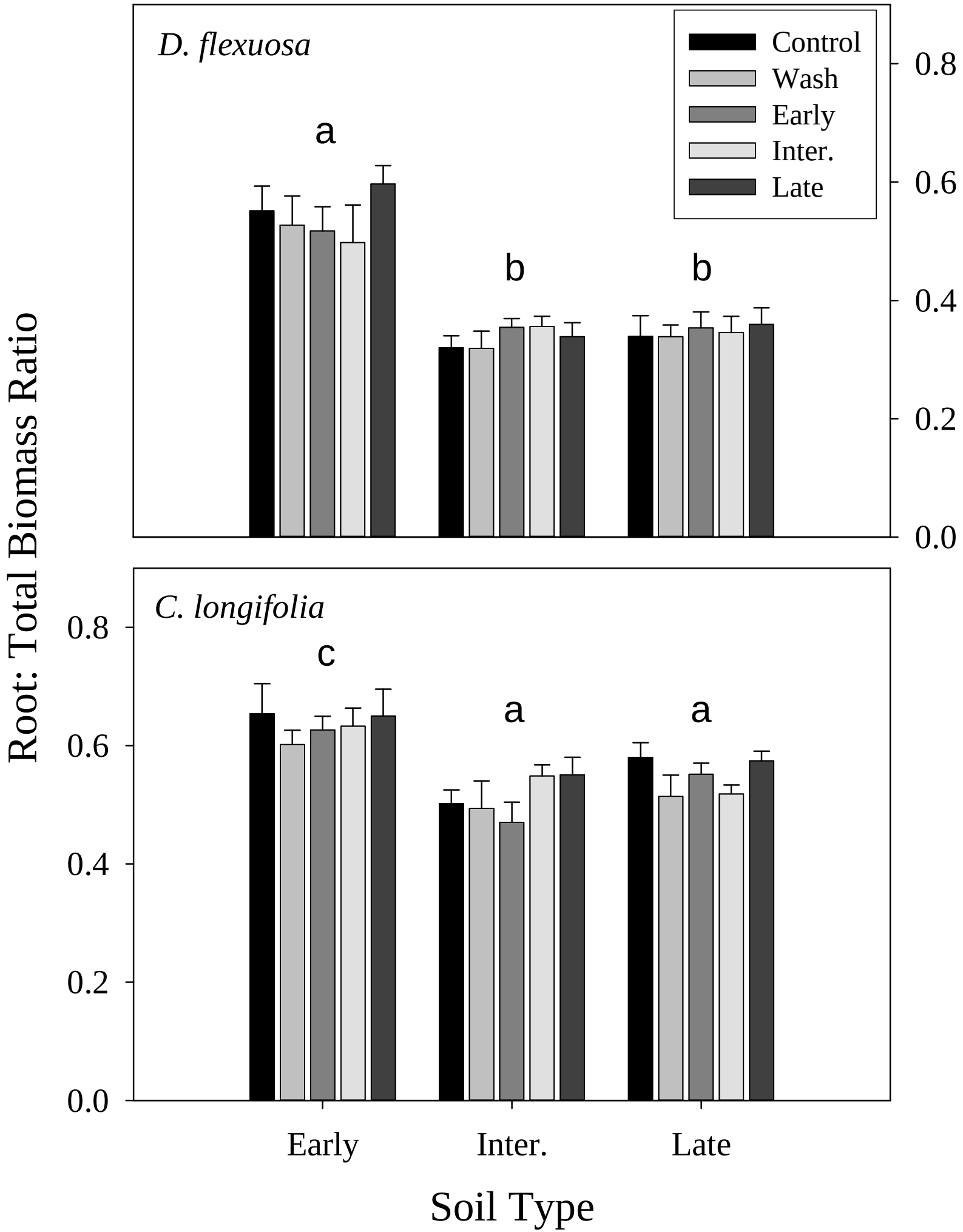

Control
Wash
Early
Inter.
Late
D. flexuosa
C. longifolia
Total Biomass (g)
0
2
4
6
Early
Inter.
Late
Soil Type
Soil
a
b
c

Fig4


D. flexuosa
Control
Wash
Early
Inter.
Late
a
b
b
0.8
0.6
0.4
0.2
0.0
C. longifolia
c
a
a
0.8
0.6
0.4
0.2
0.0
Root: Total Biomass Ratio
Early
Inter.
Late
Soil Type

Mycorrhiza Supplmentary Materials

Mycorrhizal fungal growth responds to soil characteristics, but not plant host identity, during a primary lacustrine sand dune succession

Benjamin A. Sikes[1†], Hafiz Maherali[1] and John N. Klironomos[3]

[1]Department of Integrative Biology, University of Guelph, ON, Canada N1G 2W1

[2]Department of Biology, The University of British Columbia – Okanagan, Kelowna, BC , Canada V1V 1V7
†corresponding author: Benjamin A. Sikes
current address: Department of Ecology and Evolutionary Biology and Kansas Biological Su
e-mail: ben.sikes@ku.edu
telephone: +785-864-1920
fax: +785-864-1534

Soil Variables Among Successional Stages

Variable	dF	F-value	P-value
pH	2,6	5558	**1.57E-10**
P	2,6	337.28	**6.85E-07**
Mg	2,6	76.121	**5.45E-05**
K	2,6	670.88	**8.82E-08**
C	2,6	221.09	**2.40E-06**
NO_3	2,6	1968.6	**3.52E-09**
NH_4	2,6	73.861	**5.95E-05**

Mycorrhizal Traits Among AMF communities, soil types and plant hosts

Arbuscules

	Df	Sum Sq	Mean Sq	F-value	Pr(>F)	Sign.
microbe	2	587.7	293.83	3.5973	0.0302	*
soil	2	2743.7	1371.87	16.7954	3.33E-07	***
plant	1	96.6	96.61	1.1828	0.2788	
block	9	407.3	45.26	0.5541	0.8322	
microbe:soil	4	9486.5	2371.62	29.035	< 2.20E-16	***
microbe:plant	2	357.5	178.73	2.1881	0.1163	
soil:plant	2	120.5	60.23	0.7373	0.4804	
microbe:soil:plant	4	244.1	61.03	0.7472	0.5616	
Residuals	128	10455.2	81.68			

Vesicles

	Df	Sum Sq	Mean Sq	F-value	Pr(>F)	
microbe	2	137.6	68.778	2.3418	0.10027	
soil	2	142.2	71.104	2.421	0.09289	.
plant	1	0	0.001	0	0.9956	
block	9	366	40.664	1.3845	0.20166	

microbe:soil	4	73.5	18.369	0.6254	0.6452
microbe:plant	2	17.7	8.853	0.3014	0.7403
soil:plant	2	19.9	9.947	0.3387	0.71335
microbe:soil:plant	4	148.4	37.107	1.2634	0.28782
Residuals	128	3759.4	29.37		

Intraradical Hyphae

	Df	Sum Sq	Mean Sq	F-value	Pr(>F)	
microbe	4	1616	403.89	2.808	0.0265	*
soil	2	795	397.67	2.7648	0.06517	.
plant	1	112	111.56	0.7756	0.37944	
block	9	1141	126.83	0.8818	0.5423	
microbe:soil	8	1347	168.42	1.1709	0.31778	
microbe:plant	4	471	117.71	0.8184	0.51465	
soil:plant	2	301	150.74	1.048	0.35237	
microbe:soil:plant	8	881	110.12	0.7656	0.63347	
Residuals	221	31787	143.83			

Extraradical Hyphae

	Df	Sum Sq	Mean Sq	F-value	Pr(>F)	
microbe	4	621.73	155.433	124.4454	< 2.20E-16	***
soil	2	9.54	4.769	3.8186	0.02324	*
plant	1	0.06	0.062	0.0497	0.82383	
block	9	6.09	0.677	0.5419	0.84319	
microbe:soil	8	44.08	5.511	4.4119	5.16E-05	***
microbe:plant	4	1.65	0.411	0.3294	0.85808	
soil:plant	2	4.69	2.347	1.8791	0.15485	
microbe:soil:plant	8	1.84	0.23	0.1838	0.99298	
Residuals	253	316	1.249			

Plant Metrics Among AMF communities, soil types and plant hosts

Relative Water Content (RWC)

	Df	Sum Sq	Mean Sq	F-value	Pr(>F)	
soil	2	3380	1690	35.5896	2.40E-14	***
microbe	4	300	75	1.5796	0.18019	
plant	1	3487.4	3487.4	73.4401	1.04E-15	***
block	9	4799.8	533.3	11.2309	9.48E-15	***
soil:microbe	8	771.7	96.5	2.0314	0.04331	*
soil:plant	2	1195.3	597.6	12.5853	6.16E-06	***
microbe:plant	4	307.9	77	1.621	0.16942	
soil:microbe:plant	8	489.6	61.2	1.2889	0.24954	
Residuals	253	12014	47.5			

Total Biomass

	Df	Sum Sq	Mean Sq	F-value	Pr(>F)	
soil	2	1345.04	672.52	1096.194	< 2.20E-16	***
microbe	4	4.69	1.17	1.9114	0.109	
plant	1	2.03	2.03	3.3136	0.06989	.
block	9	22.22	2.47	4.0238	7.89E-05	***
soil:microbe	8	6.27	0.78	1.2775	0.25554	
soil:plant	2	0.56	0.28	0.4549	0.63502	
microbe:plant	4	0.97	0.24	0.3949	0.8122	
soil:microbe:plant	8	5.81	0.73	1.1838	0.30911	
Residuals	253	155.22	0.61			

Root:Total Biomass Ratio

	Df	Sum Sq	Mean Sq	F-value	Pr(>F)	
soil	2	1.45264	0.72632	75.7324	< 2.20E-16	***
microbe	4	0.07954	0.01988	2.0733	0.084779	.
plant	1	1.85073	1.85073	192.9738	< 2.20E-16	***
block	9	0.22108	0.02456	2.5613	0.007797	**
soil:microbe	8	0.04413	0.00552	0.5751	0.797976	
soil:plant	2	0.14623	0.07312	7.6237	0.00061	***
microbe:plant	4	0.01135	0.00284	0.2959	0.880485	
soil:microbe:plant	8	0.05011	0.00626	0.6532	0.732407	
Residuals	253	2.42642	0.00959			

Leaves

	Df	Sum Sq	Mean Sq	F-value	Pr(>F)	
soil	2	333	166.5	1100.894	< 2.20E-16	***
microbe	4	1.72	0.43	2.8382	0.024964	*
plant	1	894	894	5911.102	< 2.20E-16	***
block	9	2.37	0.26	1.7412	0.080274	.
soil:microbe	8	3.19	0.4	2.6333	0.008709	**
soil:plant	2	69.64	34.82	230.2329	< 2.20E-16	***
microbe:plant	4	0.51	0.13	0.848	0.495968	
soil:microbe:plant	8	1.83	0.23	1.5132	0.152896	
Residuals	250	37.81	0.15			

urvey, University of Kansas, Lawrence, KS 66047, USA

Arbuscles

Microbe:Soil	diff	lwr	upr	p adj	
Mid:Early-Early:Early	4.657449	-6.908	16.2229	0.937898	
Late:Early-Early:Early	-19.3645	-30.6101	-8.11884	9.5E-06	
Early:Mid-Early:Early	3.662401	-6.24166	13.56646	0.961888	
Mid:Mid-Early:Early	1.69076	-7.98559	11.36711	0.999777	
Late:Mid-Early:Early	1.731947	-8.05293	11.51682	0.999755	
Early:Late-Early:Early	0.999635	-8.67672	10.67599	0.999996	
Mid:Late-Early:Early	-3.28855	-12.9649	6.387806	0.977118	
Late:Late-Early:Early	20.19777	10.62066	29.77487	0	***
Late:Early-Mid:Early	-24.0219	-36.3999	-11.6439	4E-07	***
Early:Mid-Mid:Early	-0.99505	-12.1683	10.17824	0.999999	
Mid:Mid-Mid:Early	-2.96669	-13.9386	8.005263	0.994848	
Late:Mid-Mid:Early	-2.9255	-13.9933	8.142276	0.995588	
Early:Late-Mid:Early	-3.65781	-14.6298	7.314137	0.979698	
Mid:Late-Mid:Early	-7.94599	-18.9179	3.025957	0.358705	
Late:Late-Mid:Early	15.54032	4.655796	26.42484	0.000492	
Early:Mid-Late:Early	23.02685	12.18497	33.86873	0	***
Mid:Mid-Late:Early	21.05521	10.42094	31.68948	2E-07	***
Late:Mid-Late:Early	21.0964	10.36328	31.82951	3E-07	***
Early:Late-Late:Early	20.36409	9.729814	30.99836	6E-07	***
Mid:Late-Late:Early	16.07591	5.441634	26.71018	0.000168	***
Late:Late-Late:Early	39.56222	29.01817	50.10626	0	***
Mid:Mid-Early:Mid	-1.97164	-11.1757	7.232405	0.999	
Late:Mid-Early:Mid	-1.93045	-11.2485	7.387617	0.999216	
Early:Late-Early:Mid	-2.66277	-11.8668	6.541279	0.991876	
Mid:Late-Early:Mid	-6.95095	-16.155	2.2531	0.302169	
Late:Late-Early:Mid	16.53537	7.435718	25.63502	2.4E-06	***
Late:Mid-Mid:Mid	0.041187	-9.03448	9.116858	1	
Early:Late-Mid:Mid	-0.69113	-9.64969	8.267435	1	
Mid:Late-Mid:Mid	-4.97931	-13.9379	3.979255	0.711739	
Late:Late-Mid:Mid	18.50701	9.655739	27.35828	0	***
Early:Late-Late:Mid	-0.73231	-9.80798	8.343359	0.999999	
Mid:Late-Late:Mid	-5.02049	-14.0962	4.055179	0.717013	
Late:Late-Late:Mid	18.46582	9.496041	27.4356	1E-07	***
Mid:Late-Early:Late	-4.28818	-13.2467	4.670381	0.848629	
Late:Late-Early:Late	19.19813	10.34687	28.0494	0	***
Late:Late-Mid:Late	23.48631	14.63505	32.33758	0	***