

Artefacts of the pot environment on soil nutrient availability: implications for the interpretation of ecological studies

J. W. Dalling · K. Winter · K. M. Andersen ·
B. L. Turner

Received: 3 September 2012 / Accepted: 16 January 2013 / Published online: 31 January 2013
© Springer Science+Business Media Dordrecht 2013

Abstract Pot experiments are frequently used to examine plant, soil, and microbial interactions that cannot be studied in situ. Although impacts of the pot environment on seedling growth have long been recognized, they are rarely addressed directly in current ecological studies. Here we examine how commonly used soil conditioners, often necessary to maintain adequate drainage in pots, can influence nutrient availability and seedling growth. Balsawood (*Ochroma pyramidalis*) seedlings were grown in central Panama in soil combined in a 50:50 mix with one of the following soil conditioners: marine sand, pure sand, perlite, vermiculite, or rice husks. Soil nutrient availability, foliar N and P, and seedling growth were compared after 1 month. Rice husks dramatically reduced soil nitrate, seedling growth, and foliar N. Other conditioners had smaller effects on growth, although vermiculite may be a significant source of base cations in infertile soils. Marine sand had a strong neutralizing effect on acidic soil due to the presence of calcareous shell fragments.

Effects of soil conditioners need to be added to other known artifacts of the pot environment influencing nutrient availability, including alterations to N mineralization rates relative to bulk soil, and pot-size effects on N:P availability. These artifacts can strongly influence plant performance and therefore the interpretation of ecological studies.

Keywords Nutrient availability · Soil conditioner · Plant growth · Pot artifacts

Introduction

Over the last decade, there has been rapid growth in the number of published studies that use pot experiments. Articles in ecology and environmental science journals indexed by Web of Science that explicitly include the term ‘pot experiment’ in the abstract or keywords rose from 39 in 2000 to 127 in 2010. While many of these studies concern the effects of heavy metals and other contaminants, another large fraction (39 %) was used to explore how nutrient availability, water relations, mycorrhizal status, and plant–soil feedbacks influence plant performance. These experiments are particularly susceptible to misinterpretation due to artifacts of the pot environment that alter conditions that plants experience relative to bulk soil.

Pot experiments are attractive to ecologists because of the potential for greater control over environmental conditions and biotic interactions than is possible in the

J. W. Dalling (✉)
Department of Plant Biology, University of Illinois at
Urbana-Champaign, 265 Morrill Hall,
505 S. Goodwin Avenue, Urbana,
IL 61801, USA
e-mail: dallingj@life.illinois.edu

J. W. Dalling · K. Winter · K. M. Andersen ·
B. L. Turner
Smithsonian Tropical Research Institute,
Apartado Postal 0843-03092, Balboa, Ancon, Panama,
Republic of Panama

field. Nonetheless, enclosing soil in pots and microcosms introduces an array of artifacts associated with changes in soil structure, temperature, nutrients, soil water relations, and oxygen availability. Experiments that co-vary pot size and nutrient availability provide convincing evidence that plants respond to soil volume (McConnaughy and Bazzaz 1991) and to the presence of roots of competing plants by changing allocation to root biomass and reproductive structures (Hess and de Kroon 2007; Paul-Victor and Turnbull 2009). In addition, soil homogenization or restricted space for root growth in pots can alter root and shoot morphology (Peterson et al. 1991a; Tsakalidimi et al. 2009), physiology and response to irrigation and elevated CO₂ treatments (Peterson et al. 1991b; McConnaughy et al. 1993), total plant biomass (Poorter et al. 2012a) and potentially benefits from mycorrhizal associates (McGonigle et al. 2003).

Drainage characteristics of pots differ fundamentally from field soil, with important consequences for seedling growth. Collection and homogenization of soil used in pot experiments can disrupt aggregate structure and lead to soil compaction (Schultz et al. 2001). Furthermore, even when porosity is unaffected, much smaller pore sizes in pots can promote greater water retention in pots compared with field soil (Passioura 2006). This is because the movement of water through soil pores is determined by capillary action, with the ability of the pores to hold water determined by pore diameter and height above the free water surface (the saturated base of the pot or the water table in field soil). As pots tend to be short relative to the vertical distance to the water table in field soil, soil pores that would be aerated in the field are likely to be water-filled in small pots.

Water logging of soil pores is problematic because roots are sensitive to hypoxia (Letey et al. 1962; Evans et al. 2009). Growth rates can be affected in soils with <10 % air filled porosity (da Silva et al. 1994)—conditions that can occur in small pots filled with field soil. Furthermore, hypoxia may be exacerbated if frequent watering destroys soil aggregate structure (Firbank et al. 1990). A common horticultural solution to soil hypoxia is to use peat, characterized by large pore spaces, as a growth medium. However, if the objective is to explore the effects of field soil on plant growth, then pore size can be increased by mixing field soil with soil conditioners such as sand, vermiculite, or perlite.

Although amending a soil can relieve problems of hypoxia in pots, soil conditioners can introduce additional problems by directly or indirectly modifying soil

nutrient availability. For example, diluting soil with marine or river sand will generally increase porosity, but calcium carbonate from shell fragments may have unintended effects by raising soil pH, unless the sand is thoroughly acid-washed. In turn, alteration of pH can affect base cation and P availability, Al and Mn toxicity, and microbial community composition (Fierer and Jackson 2006; Brady and Weil 2008). Pure sand generally has minimal cation exchange capacity (CEC) and little effect on nitrate retention in soil (Curtin and Smillie 1981), but will still affect nutrient availability via a soil dilution effect (Metcalf and Grubb 2002). Other soil conditioners may have large direct effects. Vermiculite has a very high CEC (80–150 cmol_c kg⁻¹), and some sources contain calcium and magnesium carbonates that can neutralize soil acidity (Nelson 1969; Brooking 1976). Vermiculite also has a strong capacity to adsorb ammonium ions (Shen et al. 1997). Perlite has a much lower CEC (1.5–3.5 cmol_c kg⁻¹; Bunt 1988), but both Al and base cations (Ca and K) can be mobilized from perlite when exposed to organic acids produced by roots and mycorrhizas, leading to an accumulation in plant tissue (Hobbie et al. 2009).

Horticulturalists and, more recently, ecologists have used rice husks (hulls) or coconut coir as an inexpensive and light weight conditioner to improve soil aeration in pots (Tsakalidimi 2006; Cernusak et al. 2007; Landis and Morgan 2009). The high C:N ratio of these soil amendments, however, may influence N availability to plants via microbial immobilization. For example, when compared with perlite-amended soil, the addition of rice husks reduced nitrate concentrations by up to 40 % (Evans and Gachukia 2004). Studies of fertilized potting mixes, however, suggest that perlite or vermiculite can be substituted by rice husks without impacting plant growth (Tsakalidimi 2006; Aklibasinda et al. 2011).

The aim of this study was to use a pot experiment to compare directly how a variety of soil conditioners commonly used by ecologists influence soil nutrient availability, seedling growth, and foliar nutrition of seedlings grown in pots. We also place these effects in the context of other known artifacts of pots and soil microcosms that influence nutrient availability.

Materials and methods

Plants were grown in soil mixed with six different soil conditioners at the Santa Cruz Experimental Field

Facility of the Smithsonian Tropical Research Institute in Gamboa, Panama (9°07'N, 79°42'W). A relatively fertile soil was collected from the top 10 cm of a nearby orchard, air-dried (~27 °C) and passed through a 10 mm sieve. Soil was then combined in a 50:50 mix by volume with one of the following conditioners: (a) rice husks; (b) unfertilized perlite (Midwest Perlite Inc., Appleton, Wisconsin, USA); (c) vermiculite (Hoffman Horticultural Vermiculite, Good Earth Inc., Lancaster, New York, USA); (d) rinsed marine sand (Geo F Novey Inc, Panama); (e) pure sand (US Silica, Frederick, Maryland, USA); (f) fertilized perlite containing urea, ammonium phosphate, and potassium nitrate (Miracle-Gro Perlite, The Scotts Company LLC, Marysville, Ohio). We did not include a 'soil-only' treatment because soil compaction of unconditioned forest soil severely restricts seedling growth (i.e., it does not provide a useful comparison to field conditions). Marine sand was thoroughly rinsed to remove silt, but retained small sand-sized shell fragments. The nutrient content of the soil (after collection) and of each of these conditioners was measured prior to the experiment.

For each of the soil mixes, we filled six 24 cm tall, 10 cm wide, 1.65 L treepots (TP49; Stuewe and Sons, Tangent, Oregon, USA), and transplanted into each pot three, 1 week old seedlings of the tree *Ochroma pyramidale* (balsawood) collected from seed on nearby Barro Colorado Island. Seedlings were allowed to establish in the pot for 1 week and then thinned to one seedling per pot. Pots were grown in full sun in an open growing house with a glass roof. Seedlings were watered daily. The initial dry mass of six randomly selected seedlings was determined at the time of transplant. Seedlings were grown for 32 days for all except the perlite treatment (38 days). At harvest time, seedlings were gently washed from the pots, separated into root, leaf, and stem fractions, leaf area measured with an automated leaf area meter (LI-3000A, LICOR, Lincoln, Nebraska) and final biomass measured after drying for 72 h at 70 °C. Total plant biomass at harvest was used to calculate the total plant biomass per unit of soil volume [biomass volume ratio BVR; (Kerstiens and Hawes 1994; Poorter et al. 2012a)]. Relative growth rate (RGR; $\text{mg}^{-1} \text{g}^{-1} \text{day}^{-1}$) was calculated as the slope of the relationship between \ln total biomass and time between seedling transplant and harvest for each soil treatment. Root mass ratio

(RMR; root mass per unit whole plant biomass) and Specific leaf area (SLA; leaf area per unit leaf mass; $\text{cm}^{-2} \text{g}^{-1}$) were calculated from harvest data. Finally, leaf material from each harvested seedling was ground and analyzed for total N and P (see below).

We filled an additional 20 pots with each of the six soil mixtures to track changes in soil nutrients and soil pH over the course of the experiment in the absence of seedlings. For all treatments (except unfertilized perlite), we collected soil from four replicate pots 0, 1, 2, 4, and 7 weeks after seedlings were transplanted.

Analysis

Ammonium and nitrate concentrations were determined by extraction in 2 M KCl by automated colorimetry using a Lachat Quickchem 8500 (Hack Ltd, Loveland, Co, USA). Soil pH was determined in deionized water in a 1:2 soil to solution ratio. Readily exchangeable phosphate was extracted by anion exchange membranes (551642S, BDH-Prolabo, VWR International, Lutterworth, UK), elution of the phosphate in 0.25 M H_2SO_4 , and detection by automated molybdate colorimetry (Turner and Romero 2009). Microbial P was estimated as the phosphate released to the membrane strips by hexanol fumigation (Turner and Romero 2010). Exchangeable cations were determined by extraction in 0.1 M BaCl_2 with detection by inductively coupled plasma optical emission spectrometry (Optima 7300DV; Perkin Elmer, Shelton, CT, USA) (Hendershot et al. 2008). Effective cation exchange capacity (ECEC) was calculated as the sum of cations (Al, Ca, Fe, K, Mg, Mn, Na).

For seedlings, foliar nitrogen concentration was measured by automated combustion and thermal conductivity detection on a Thermo Flash EA112 analyzer (CE Elantech, New Jersey, USA), and foliar phosphorus by ignition (550 °C, 1 h) and acid extraction (1 M H_2SO_4 , 16 h) with phosphate detection by automated molybdate colorimetry on a Lachat Quickchem 8500 (Hach Ltd, Loveland, Colorado, USA).

Differences in soil nutrient availability (expressed on a volumetric basis to control for differences in soil bulk density), and in seedling growth and allocation patterns were analyzed using fixed factor ANOVA. Whereas the main treatment effect was significant, differences among conditioners were assessed using Tukey HSD (Crawley 2007).

Results

Analysis of common soil conditioners indicated that they could potentially have strong effects on soil fertility (Table 1). In particular, rice husks appear to significantly increase plant-available P and to a lesser extent K despite their low bulk density. Similarly, vermiculite may enhance base cation availability due to high concentrations of Ca, K, and Mg, and marine sand may be an important source of Ca and K. Contributions to N, P, and cations from pure sand and perlite were negligible.

Mixing conditioners with soil can further alter nutrient availability over time. For all soil conditioners, there was a strong decline in extractable ammonium during the experiment, reaching $<0.3 \mu\text{g cm}^{-3}$ after 7 weeks (Table 2; Fig. 1a). In contrast, nitrate concentrations remained relatively high throughout the experiment with the exception of the rice husk treatment where values dropped to $0.4 \mu\text{g cm}^{-3}$ after 4 weeks, resulting in strong N limitation (Table 2; Fig. 1b). In addition to effects on N availability, soil amendments also had significant effects on soil pH and base cation availability (Table 2). Marine sand contains calcareous shell fragments which neutralized the pH of the soil over 7 weeks. The other notable effect was an increase in K availability in the vermiculite treatment (Table 2).

Differences in soil nutrient availability resulted in significant treatment effects on seedling growth and allocation (Fig. 2). At the time of harvest, total plant biomass of *Ochroma* seedlings ranged between 1.30 g

(SE \pm 0.07) in the fertilized perlite treatment and 0.10 g (SE \pm 0.01) in the rice treatment, corresponding to a maximum total plant biomass: rooting volume ratio (BVR) of 0.79 g L^{-1} . Low final biomass in the rice husk-conditioned soil, and correspondingly reduced RGR (Figs. 2, 3a), reflected the observation that plants stopped producing new leaves after 4 weeks in this treatment. Differences in RGR among the remaining treatments were much smaller, but seedlings grew significantly more slowly in the perlite than the sand treatments. RGR was highest in the fertilized perlite treatment, indicating some nutrient limitation to growth in the soil used. Strong N limitation in the rice husk-conditioned soil was also reflected in below-ground allocation; RMR in the rice husk treatment was nearly twice that in the perlite and vermiculite treatments. RMR was also significantly higher in the two sand treatments compared to the vermiculite and perlite treatments (Fig. 3b). Treatment effects on leaf-level allocation patterns were also evident, with significantly lower SLA in the rice husk treatment, and higher SLA in the perlite treatment (Fig. 3c).

Differences in growth rate were also reflected in significant treatment effects on foliar N, P, and N:P (Fig. 4). Strong N limitation in the rice husk treatment resulted in foliar N concentrations that were half that of remaining treatments (Fig. 4a). Significant differences in foliar N among the remaining treatments were not associated with treatment differences in RGR or RMR, and therefore probably do not reflect N limitation. Instead, lower foliar P (Fig. 4b) and higher foliar N:P (Fig. 4c) suggest potential P limitation.

Table 1 Chemical properties of the bulk soil and pure samples of the soil conditioners used in the pot experiment

Variable	Bulk soil ^a	Rice husks	Perlite	Vermiculite	Marine sand	Pure sand	Perlite + fertilizer
NH ₄ (mg N kg ⁻¹)	11.08	11.03	0.55	3.45	0.18	0.03	72.54
NO ₃ (mg N kg ⁻¹)	51.43	3.21	0.60	0.79	0.41	0.23	346.82
Extractable PO ₄ (mg P kg ⁻¹)	17.80	109.2	0.01	2.81	0.71	0.23	85.77
Al (cmol _c kg ⁻¹)	0.41	0.01	0.04	0.07	0	0	0.01
Ca (cmol _c kg ⁻¹)	30.60	1.61	0.14	4.88	0.56	0.09	0.90
K (cmol _c kg ⁻¹)	1.64	5.22	0	4.10	0.61	0	0.41
Mg (cmol _c kg ⁻¹)	20.4	1.9	0	36.3	0.3	0	0.60
Mn (cmol _c kg ⁻¹)	0.1	0.8	0	0.3	0	0	0
Na (cmol _c kg ⁻¹)	0.1	0.2	0.20	0.7	0.1	0	1.3
ECEC (cmol _c kg ⁻¹)	53.2	9.7	0.40	46.3	1.6	0.2	3.2

ECEC effective cation exchange capacity

^a After sieving

Table 2 Bulk density, and mean and standard deviation of extractable nutrients in each of the soil treatments 7 weeks after initiating the experiment

Variable	Effect size (<i>F</i>)	Rice husks	Perlite	Vermiculite	Marine sand	Pure sand	Perlite + fertilizer
Bulk density (g cm ⁻³)	–	0.54	0.60	0.57	1.24	1.24	0.60
pH	802.2***	5.67 ^c (0.05)	5.55 ^b (0.11)	5.47 ^b (0.03)	7.04 ^d (0.08)	5.52 ^b (0.02)	5.17 ^a (0.03)
NH ₄ -N (μg cm ⁻³)	2.1	0.16 (0.05)	0.32 (0.07)	0.23 (0.06)	0.26 (0.07)	0.22 (0.04)	0.24 (0.02)
NO ₃ -N (μg cm ⁻³)	148.1***	0.40 ^a (0.14)	30.3 (7.3)	32.83 ^c (5.55)	13.58 ^b (4.78)	24.56 ^c (3.79)	56.23 ^d (21.02)
Resin P (μg cm ⁻³)	36.0***	9.6 ^c (3.7)	7.4 ^b (0.6)	5.3 ^{a,b} (0.5)	4.3 ^a (0.2)	3.8 ^a (0.3)	16.4 ^d (1.2)
Microbial P (μg cm ⁻³)	18.7***	6.6 ^b (2.1)	–	1.4 ^a (0.6)	1.9 ^a (0.6)	1.3 ^a (0.6)	–
Al (μg cm ⁻³)	148.6***	25.3 ^b (1.2)	16.6 ^a (2.3)	20.0 ^a (1.7)	20.0 ^a (0.8)	46.9 ^d (2.1)	32.2 ^c (2.4)
Ca (μg cm ⁻³)	11.3***	4100 ^{a,b} (179)	4704 ^b (172)	3986 ^{a,b} (223)	4379 ^b (646)	3433 ^a (391)	3303 ^a (166)
K (μg cm ⁻³)	64.1***	521 ^b (54)	364 ^a (18)	960 ^c (116)	354 ^d (71)	372 ^a (43)	317 ^a (18)
Mg (μg cm ⁻³)	13.8***	1681 ^b (78)	1764 ^b (65)	2102 ^b (156)	1585 ^{a,b} (259)	1446 ^a (162)	1350 ^a (48)
Mn (μg cm ⁻³)	38.0***	6.2 ^{b,c} (1.2)	2.6 ^a (0.4)	5.0 ^b (0.2)	5.8 ^b (0.2)	7.7 ^c (0.5)	5.9 ^b (0.4)
Na (μg cm ⁻³)	18.0***	34.7 ^a (5.2)	67.1 ^{b,c} (7.2)	54.0 ^{a,b} (10.9)	84.9 ^c (14.8)	50.1 ^{a,b} (6.6)	79.5 ^c (6.1)
ECEC (cmol _c L ⁻¹)	9.7***	36.1 ^{a,b} (1.7)	39.4 ^b (1.5)	40.1 ^b (2.6)	36.4 ^b (5.6)	30.8 ^{a,b} (3.4)	29.1 ^a (1.3)

Effect sizes are from the ANOVA of treatment effects (df 5, 19). Superscript letters indicate significant differences among soil treatments ($P < 0.05$) where an overall treatment effect was significant. Nitrate and ammonium data for perlite were only available for week 2 and are excluded from analyses. Microbial P could not be measured for soil perlite mixes

*** $P < 0.001$

Discussion

Results of the bioassay experiment indicate that soil conditioners can have substantial impacts on nutrient availability, and consequently seedling growth and allocation. These effects arise from (a) a neutralizing effect of carbonate present in marine sand, (b) contributions to N availability and cation exchange capacity from vermiculite addition, (c) increased plant-available P from rice husks, and (d) strong microbial immobilization of soil nitrogen resulting from adding organic matter with a high C:N ratio in rice husks.

Sand is a commonly used and readily available soil conditioner that has minimal direct effects on soil nutrient availability. A potential neutralizing effect of calcium carbonate present as shell fragments in marine sand may be overlooked by investigators. This can be avoided by acid washing sand prior to use, though this may make alternative commercial conditioners more cost-effective to use. Of the two commercial conditioners we tested, perlite contributed little to soil nutrient availability, while vermiculite could be a significant source of N and base cations when added to infertile soil. Nitrogen limitation can limit plant

growth across multiple biomes (LeBauer and Treseder 2008), so changes in N availability should be a key concern in designing pot experiments. Evidence that base cation availability limits plant growth is scarce, although a recent fertilization experiment in lowland tropical forest has shown that K addition influences fine root biomass (Wright et al. 2011) and seedling growth and root mass ratio (Santiago et al. 2011). The use of vermiculite which could provide a significant source of K in some soils should therefore be exercised with caution.

The most striking result of the pot experiment was the strong negative effect of rice husks on nitrate availability (Fig. 1), resulting in marked N limitation to seedling growth (Figs 2, 3a). The decline in N availability in this treatment reflects immobilization of inorganic N by decomposer organisms that use the rice husks as a carbon source. The results of this experiment are likely to be general to all organic soil conditioners with a high C:N, and mirrors results of field experiments using carbon-rich mulch treatments (Sinha et al. 1977). Results here are also consistent with strong N limitation observed in other pot experiments using rice husks (Cernusak et al. 2007,

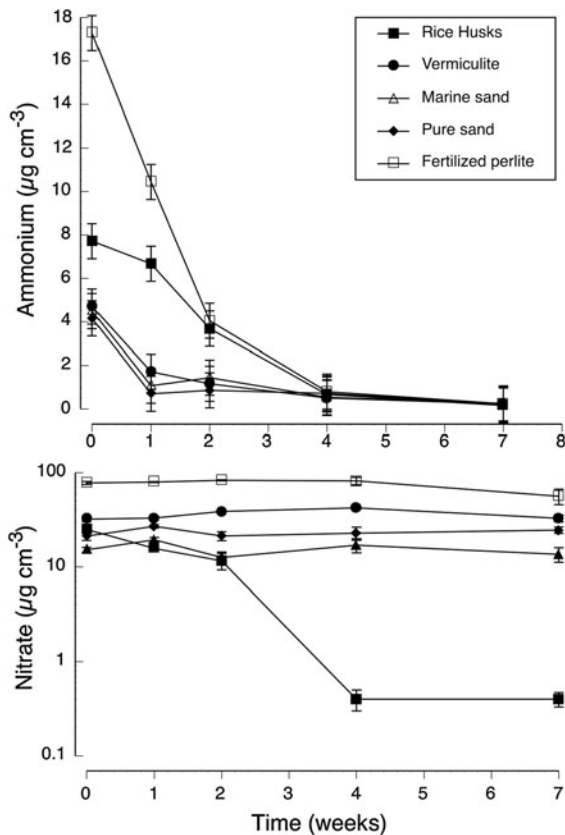


Fig. 1 Changes in **a** extractable $\text{NH}_4\text{-N}$ and **b** extractable $\text{NO}_3\text{-N}$ (log scale) over 7 weeks in pots without seedlings containing a 50:50 mix of forest soil and one of five different soil conditioners. Values are means of four pots (± 1 SE)

2011). Nonetheless, the ability of rice husks to immobilize nitrate may be an advantage in some contexts. Further experiments could explore whether addition of rice husks to soil can be used to create a range of stable soil N:P or N:cation treatments.

Soil conditioner effects in context

Plant pots and microcosms can induce additional artifacts, influencing seedling performance that is seldom discussed in recent ecological literature (but see Poorter et al. 2012a, b). Wiersum (1962) showed that the structure of rooting substrates influences root morphology and influences the capacity to take up nitrogen and phosphorus. This may be of relevance to the choice of conditioners because containers filled with coarse aggregates supported less fine root growth of crop plants than those containing finer aggregates of the same material. In turn, coarse roots explored less of the substrate volume and were therefore less able to take up relatively immobile phosphate ions compared to mobile nitrate ions that move more freely through soil solution. As a consequence, plants grown in coarse aggregate media had much higher N:P ratios in their tissue.

Cornforth (1968) extended this work to show that pot size could have an effect much like aggregate size. In small pots, where the ratio of soil volume to root surface area is low, plants became limited by nitrogen,



Fig. 2 *Ochroma pyramidale* seedlings at harvest time in three of the six soil conditioner treatments. *Left side* seedlings grown with pure sand, *Center* seedlings grown with rice husks, *Right side* seedlings grown with fertilized perlite

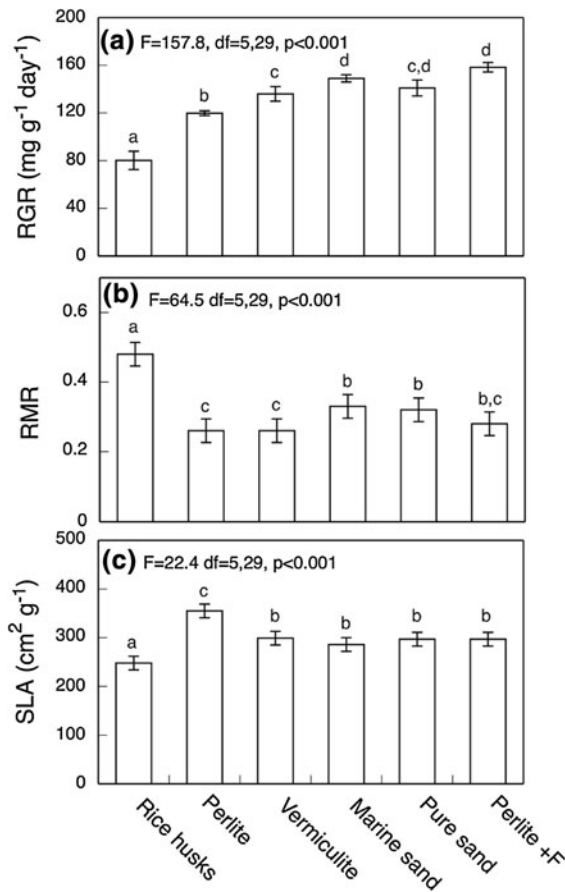


Fig. 3 Variation in relative growth rate (RGR), root mass ratio (RMR), and specific leaf area (SLA) of *Ochroma pyramidale* seedlings grown in pots containing a 50:50 mix of forest soil and one of six soil conditioners. Values are means of six pots (± 1 SE)

whereas in large pots, where a large soil volume contributes more nitrate ions to plant roots, plants can become limited by their access to phosphate. Furthermore, relative N and P limitation is a dynamic process in pots as plant roots fill the media. Peace and Grubb (1982) showed a switch from P limitation early in seedling growth to N limitation as plants matured. This size-dependent effect on nutrient limitation could also be expressed by altering plant density (N limitation at high density; P limitation at low density).

Although nitrate immobilization was observed in the rice husk treatment, pot environments are also expected to induce large increases in nitrate availability. Nitrate production can be enhanced by soil disturbance, e.g., through tillage (Kristensen et al. 2000) or when soil is placed inside cores (Ross and Hales 2003; Booth et al.

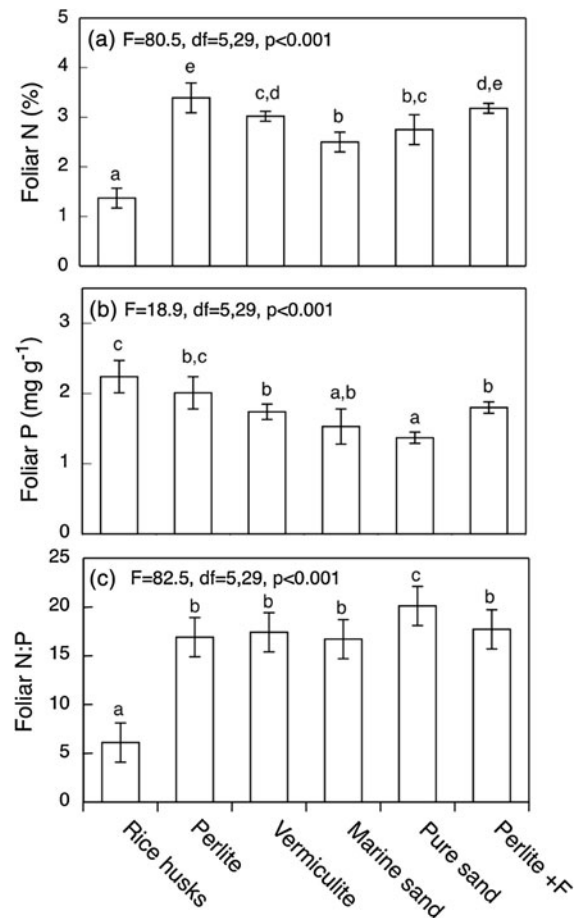


Fig. 4 Foliar N, P, and N:P of *Ochroma pyramidale* seedlings grown in pots containing a 50:50 mix of forest soil and one of six soil conditioners. Values are means of six pots (± 1 SE)

2006). Simply collecting soil samples for later chemical analysis can also result in considerable increases in ammonium and nitrate concentrations within hours of removal from the field (Turner and Romero 2009). Effects are therefore also expected when soil is collected and homogenized for use in pot experiments. We lack data on the in field nitrate availability of the soil used in our pot experiment. Nonetheless, nitrate concentrations measured using a KCl or K₂SO₄ extraction assay done in the field have shown that instantaneously extractable nitrate concentrations are $< 5 \mu\text{g N g}^{-1}$ for three lowland tropical forest soils in central Panama (Turner and Romero 2009), while extracts from other Panama soils are frequently below detectable limits (BL Turner, unpublished data). The high nitrate pool size ($51 \mu\text{g N g}^{-1}$), we measured in the bulk soil used in this experiment therefore represents an additional pot artifact.

Despite the magnitude of pot and conditioner effects on N availability, very few studies have interpreted the outcome of pot or mesocosm experiments in the context of altered N supply. Johnson et al. (1995) set up paired field (open chamber) and pot experiments that explored N fertilization and elevated CO₂ effects on ponderosa pine (*Pinus ponderosa*) seedlings. At all CO₂ levels, N fertilization had positive effects on biomass growth in the field experiment, but negative effects on biomass growth in pots. Lysimeter measurements revealed that differences in growth response were related to soil solution nitrate concentrations in unfertilized pots, which were more than two orders of magnitude higher than those in the field.

Conclusions and recommendations

Although we recognize that pots can never completely replicate the microbial environment or resource availability of field soil, they, nonetheless, remain an important tool for exploring plant responses to treatment manipulations that are not feasible in undisturbed systems. Two recent reviews have highlighted the importance of soil volume in determining plant growth responses in pots (Poorter et al. 2012a) and have provided an overview of how an array of environmental factors vary in controlled experiments (Poorter et al. 2012b). Our experiment extends this work by highlighting how commonly used soil conditioners can influence soil nutrient availability in ways that may strongly influence the outcome of pot experiments, either by substantially increasing availability of soil nutrients, or by immobilizing soil nitrogen. Based on these and earlier reports of pot artifacts, we suggest that the following practices should be used where possible:

Standardize pot size and shape

Pot size and shape influence both the relative availability of potentially limiting soil nutrients and the fraction of unsaturated soil pore space (Peace and Grubb 1982; Passioura 2006; Poorter et al. 2012a). To minimize these effects, we recommend using pots with a large height to volume ratio and, where possible, to standardize pot sizes across experiments. Studies should report the dimensions as well as the volume of pots used.

Measure plant traits at a standard size

Another means of minimizing the effects of differential pot volume on plant growth and allocation is to standardize plant size at transplant and at harvest. For some experiments, it may be more important to standardize plant size across treatments than to standardize experiment duration. Based on a meta-analysis of plant growth responses to pot size, Poorter et al. (2012a, b) further suggest that the plant biomass to soil volume ratio (BVR) at harvest should not exceed 1 g L⁻¹ to prevent constraints of pot volume on plant growth.

Match soil conditioners to soil nutrient availability

The use of conditioners to maintain open pore space may be unavoidable when using field soil with fine aggregate structure. Most conditioners will not influence nutrient availability except in the most infertile soils. However, care should be taken to avoid the neutralizing effect of carbonates in sand, and the nitrogen immobilizing effect of organic amendments. At a minimum, extractable inorganic N should be measured at the completion of the experiment.

Acknowledgments We thank Milton Garcia, Jorge Aranda, Aurelio Virgo, and Tania Romero for technical assistance in carrying out the experiment, and the Smithsonian Tropical Research Institute for financial and logistic support.

References

- Aklibasinda M, Tunc T, Bulut Y, Sahin U (2011) Effects of different growing media on scotch pine production. *J Anim Plant Sci* 21:535–541
- Booth MS, Stark JM, Hart SC (2006) Soil-mixing effects on inorganic nitrogen production and consumption in forest and shrubland soils. *Plant Soil* 289:5–15
- Brady NC, Weil RR (2008) The nature and properties of soils, 14th edn. Pearson Prentice Hall, New Jersey
- Brooking IR (1976) Soilless potting media for controlled environment facilities. *New Zeal J Exp Agr* 4:203–208
- Bunt AC (1988) Media and mixes for container grown plants. Unwin Hyman, London
- Cernusak LA, Winter K, Aranda J, Turner BL (2007) Transpiration efficiency of a tropical pioneer tree (*Ficus insipida*) in relation to soil fertility. *J Exp Bot* 58:3549–3566
- Cernusak LA, Winter K, Martínez C, Correa E, Aranda J, Garcia M, Jaramillo C, Turner BL (2011) Responses of legume versus non-legume tropical tree seedlings to elevated [CO₂]. *Plant Physiol* 157:372–385

- Cornforth IS (1968) Relationship between soil volume used by roots and nutrient accessibility. *J Soil Sci* 19:291–301
- Crawley MJ (2007) *The R book*. Wiley, Chichester
- Curtin D, Smillie GW (1981) Contribution of the sand and silt fractions to the cation exchange capacities of some Irish soils. *J Earth Sci* 4:17–20
- da Silva AP, Kay BD, Perfect E (1994) Characterization of the least limiting water range of soils. *Soil Sci Soc Am J* 58:1775–1781
- Evans MR, Gachukia M (2004) Fresh parboiled rice hulls serve as an alternative to perlite in greenhouse crop substrates. *HortScience* 39:232–235
- Evans RY, Hansen J, Dodge LL (2009) Growth of rose roots and shoots is highly sensitive to anaerobic or hypoxic regions of container substrates. *Sci Hortic* 119:286–291
- Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci USA* 103:626–631
- Firbank LG, Cousens R, Mortimer AM, Smith RGR (1990) Effects of soil type on crop yield-weed density relationships between winter wheat and *Bromus sterilis*. *J Appl Ecol* 27:308–318
- Hendershot WH, Lalonde H, Duquette M (2008) Ion exchange and exchangeable cations. In: Carter MR, Gregorich E (eds) *Soil sampling and methods of analysis*. Canadian Society of Soil Science, CRC Press, Boca Raton, pp 173–178
- Hess L, de Kroon H (2007) Effects of rooting volume and nutrient availability as an alternative explanation for root self/non-self discrimination. *J Ecol* 95:241–251
- Hobbie EA, Hoff CJ, Bryce JG, Colpaert JV, Hallett RA (2009) Nutrient supply rate and mycorrhizal colonization control patterns of element distribution in ectomycorrhizal pine. *Commun Soil Sci Plant Anal* 40:3503–3523
- Johnson DW, Walker RF, Ball TJ (1995) Lessons from lysimeters: soil N release from disturbance compromises controlled environment study. *Ecol Appl* 5:395–400
- Kerstiens G, Hawes C (1994) Response of growth and carbon allocation to elevated CO₂ in young cherry (*Prunus avium* L.) saplings in relation to root environment. *New Phytol* 128:607–614
- Kristensen HL, McCarty GW, Meisinger JJ (2000) Effects of soil structure disturbance on mineralization of organic soil nitrogen. *Soil Sci Soc Am J* 64:371–378
- Landis TD, Morgan N (2009) Growing media alternatives for forest and native plant nurseries. USDA For Serv Proc RMRS-P-58, p 26–31
- LeBauer DS, Treseder KK (2008) Nitrogen limitation of primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89:371–379
- Letej J, Stolzy LH, Blank GB (1962) Effect of duration and timing of low oxygen content on shoot and root growth. *Agron J* 54:34–57
- McConaughy KDM, Bazzaz FA (1991) Is physical space a soil resource? *Ecology* 72:94–103
- McConaughy KDM, Berntson GM, Bazzaz FA (1993) Limitations to CO₂ induced growth enhancement in pot studies. *Oecologia* 94:550–557
- McGonigle TP, Yano K, Shinhama T (2003) Mycorrhizal phosphorus enhancement of plants in undisturbed soil differs from phosphorus uptake stimulation by arbuscular mycorrhizae over non-mycorrhizal controls. *Biol Fertil Soils* 37:268–273
- Metcalfe DJ, Grubb PJ (2002) Soil dilution as a surrogate for root competition: effects on growth of seedlings of Australian tropical rainforest trees. *Funct Ecol* 16:223–231
- Nelson PV (1969) Assessment and correction of the alkalinity problem associated with Palabora vermiculite. *J Am Soc Hort Sci* 94:664–667
- Passioura JB (2006) The perils of pot experiments. *Funct Plant Biol* 33:1075–1079
- Paul-Victor C, Turnbull LA (2009) The effect of growth conditions on the seed size/number trade-off. *PLoS ONE* 4:e6917
- Peace WJH, Grubb PJ (1982) Interaction of light and mineral nutrient supply in the growth of *Impatiens parviflora*. *New Phytol* 90:127–150
- Peterson TA, Reinsel MD, Krizek DT (1991a) Tomato (*Lycopersicon esculentum* Mill cv. 'Better Bush') plant response to root restriction. Alteration of plant morphology. *J Exp Bot* 42:1233–1240
- Peterson TA, Reinsel MD, Krizek DT (1991b) Tomato (*Lycopersicon esculentum* Mill cv. 'Better Bush') plant response to root restriction. Root respiration and ethylene generation. *J Exp Bot* 42:1241–1249
- Poorter H, Bühler J, van Dusschoten D, Climent J, Postma JA (2012a) Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Funct Plant Biol*. doi: [10.1071/FP12049](https://doi.org/10.1071/FP12049)
- Poorter H, Fiorani F, Stitt M, Schurr S, Fink A, Gibon Y, Usadel B, Munns R, Atkin O, Tardieu F, Pons TL (2012b) The art of growing plants for experimental purposes: a practical guide for the plant biologist. *Funct Plant Biol*. doi: [10.1071/FP12028](https://doi.org/10.1071/FP12028)
- Ross DS, Hales HC (2003) Sampling-induced increases in net nitrification in the brush brook (Vermont) watershed. *Soil Sci Soc Am J* 67:318–326
- Santiago LS, Wright SJ, Harms KE, Yavitt JB, Korine C, Garcia MN, Turner BL (2011) Tropical tree seedling growth responses to nitrogen, phosphorus and potassium addition. *J Ecol* 100:309–316
- Schultz PA, Miller MR, Jastrow JD, Rivetta CV, Bever JD (2001) Evidence of a mycorrhizal mechanism for the adaptation of *Andropogon gerardii* (Poaceae) to high- and low-nutrient prairies. *Am J Bot* 88:1650–1656
- Shen S, Tu S-I, Kemper WD (1997) Equilibrium and kinetic study of ammonium adsorption and fixation in sodium treated vermiculite. *Soil Sci Soc Am J* 61:1611–1618
- Sinha MK, Sinha DP, Sinha H (1977) Organic matter transformations in soils V. Kinetics of carbon and nitrogen mineralization in soils amended with different organic materials. *Plant Soil* 46:579–590
- Tsakalidimi M (2006) Kenaf (*Hibiscus cannabinus* L.) core and rice hulls as components of container media for growing *Pinus halepensis* M. seedlings. *Bioresour Technol* 97:1631–1639
- Tsakalidimi M, Tsitsoni T, Ganatsas P, Zagas T (2009) A comparison of root architecture and shoot morphology between naturally regenerated and container grown seedlings in *Quercus ilex*. *Plant Soil* 324:103–113
- Turner BL, Romero TE (2009) Short-term changes in extractable inorganic nutrients during storage of tropical rain forest soils. *Soil Sci Soc Am J* 73:1972–1979

- Turner BL, Romero TE (2010) Stability of hydrolytic enzyme activity and microbial phosphorus during storage of tropical rain forest soils. *Soil Biol Biochem* 42:459–465
- Wiersum LK (1962) Uptake of N and P in relation to soil structure and nutrient mobility. *Plant Soil* 16:62–70
- Wright SJ, Yavitt JB, Wurzbarger N, Turner BL, Tanner EVJ, Sayer EJ, Santiago LS, Kaspari M, Hedin LO, Harms KE, Garcia MN, Corre MD (2011) Potassium, phosphorus or nitrogen limit root allocation, tree growth and litter production in a lowland tropical forest. *Ecology* 92:1616–1625