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Impacts of compost application on the formation and functioning of arbuscular mycorrhizas

Soil Biology and Biochemistry, 2014; 78:38-44

10.1016/j.soilbio.2014.07.007

Title: Impacts of compost application on the formation and functioning of arbuscular mycorrhizas

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Abstract

With rising fertilizer costs, and concerns about the environmental impacts of excessive fertilizer use, organic matter is increasingly seen as an important source of nutrients in agriculture. This study investigated the impact of different rates of compost addition on the formation and functioning of arbuscular mycorrhizas (AM). A glasshouse experiment was conducted in which a mycorrhiza defective tomato mutant, and its mycorrhizal wild-type progenitor were grown in soil amended with a municipal greenwaste derived compost, at four rates of application. Impacts on the formation and functioning of AM were quantified. While compost addition at low levels of application had little effect on the formation of AM, at higher rates a small decrease in colonization of roots by AMF was observed. Both AM and compost application had a significant impact on

plant growth and/or nutrition. Although the formation of AM had relatively little impact on plant growth, plants grew progressively better with increasing compost supply. In contrast, the formation of AM had a strong positive effect on plant P and Zn acquisition, especially at lower rates of compost application. AM and compost can have an important role in agricultural systems, especially those that place a strong emphasis on biologically regulated nutrient supply systems.

Key Words

Arbuscular Mycorrhizas (AM); Biologically Regulated Nutrient Supply Systems (BRNSS); Compost; Tomato (*Solanum lycopersicum* L.); Plant Zinc (Zn) nutrition.

1 Introduction

With rising fertilizer costs, and concerns about the environmental impacts of excessive fertilizer use, there has been increasing scrutiny of nutrient management on farms (Tilman et al., 2002). An important part of this scrutiny has been to identify nutrient sources that can be readily and reliably used to support plant growth (Conyers and Moody, 2009; Hargreaves et al., 2008). It has long been recognized that organic amendments, such as cover crop residues and composts, among many others, contain significant amounts of nutrients than can be used to maintain and enhance plant growth (Quilty and Cattle, 2011). To this end, many farming systems rely solely, or in large part, on organic sources of nutrients. Such farming systems include organic farming, hybrid organic-conventional farming, or as is the case for most of the world's farmers, subsistence farming (Cardoso and Kuyper, 2006; Nelson and Janke, 2007; Placea et al., 2003; Watson et al., 2002).

A major challenge in the use of organic amendments is ensuring that they provide a reliable and predictable supply of nutrients (Quilty and Cattle, 2011; Rose et al., 2014). Whereas precise amounts of inorganic nutrients can be applied to the soil, and relatively accurate plant responses predicted, this is less often the case with organic amendments. This is because nutrients in organic forms need to be mineralized before they can be taken up by plants (Jackson et al., 2008; Paul, 2006). While this can provide a 'slow release' of nutrients over the course of the growing season, the actual amounts and timing of nutrient release are much less predictable than where inorganic fertilizers are used.

Composts, that is, humified organic matter produced via biologically-mediated oxidative processes (Hargreaves et al., 2008; Quilty and Cattle, 2011;

63 Zmora-Nahum et al., 2007), are one of the most commonly used organic
64 amendments in agriculture (see Quilty and Cattle, 2011, for recent review). In
65 addition to providing an important source of nutrients, they can also increase
66 soil carbon stocks, and help to improve soil structure and water retention (e.g.
67 Caravaca et al., 2002; Raviv et al., 1998). Another key benefit of composts is that
68 they can be readily made on small- or large-scales, and provide a means of
69 disposing of a wide variety of organic waste streams, many of which are
70 produced on farms. It is for this reason that composts have been identified as an
71 important option for managing nutrients on farms.
72 The capacity of plants to acquire nutrients is affected by many factors. The
73 formation of arbuscular mycorrhizas (AM), associations between the roots of
74 most terrestrial plant species and a relatively small group of soil fungi, can
75 increase the capacity of plants to acquire nutrients from the soil (Smith and
76 Read, 2008). The fungi do this by growing beyond the nutrient depletion zones
77 that typically form around roots, and by greatly increasing the absorptive surface
78 of the root system. Their rapid growth and high plasticity enables the fungi to
79 exploit nutrient patches in the soil, and to better respond to the tremendously
80 complex spatio-temporal dynamics of soil nutrients (Facelli and Facelli, 2002;
81 Tibbett, 2000). Arbuscular mycorrhizal fungi (AMF) are able to take up nutrients
82 in inorganic forms (Marschner and Dell, 1994). And, while there is some
83 evidence to suggest that AM may access nutrients from organic sources (Hodge
84 et al., 2001; Hodge and Fitter, 2010), this most likely occurs following the
85 mineralization of nutrients in organic matter (see Smith and Smith, 2011 for
86 discussion). Irrespective of the mechanisms involved, it is likely that AMF will be
87 important in helping plants to acquire nutrients released from compost.

88 Although insights have been gained into how compost addition affects the
89 formation of AM, relatively few studies have considered impacts on the
90 functioning of AM (Caravaca et al., 2003; Puschel et al., 2008; Roldan et al., 2006).
91 The formation of AM, typically measured as percent of root length colonized
92 (Smith and Read, 2008), can be adversely affected when inorganic nutrients,
93 especially P, are added to the soil in large amounts (Baon et al., 1992; Bolan et al.,
94 1984; Watts-Williams and Cavagnaro, 2012). For example, for the mycorrhizal
95 tomato genotype used in this study and grown in the same soil (see below),
96 percent root length colonized was reduced from 85% through 60% to 40% when
97 soil P was increased from 4, 20 and 76 mg plant-available P/kg dry soil. Effects of
98 P supply on the formation of AM are especially relevant to farming systems
99 where large amounts of inorganic fertilizer are added to the soil. In contrast,
100 since composts (typically) provide a sustained release of nutrients over the
101 course of a growing season (or seasons), rather than a large single pulse of
102 nutrients, they may offer a way of supplying nutrients to plants that does not
103 adversely affect the formation of AM. This, however, has not been widely
104 assessed, and results are inconsistent, with compost addition resulting in an
105 increase, a decrease, or no change in mycorrhizal colonization of roots
106 (e.g.Caravaca et al., 2003; Duong et al., 2012; Puschel et al., 2008; Roldan et al.,
107 2006). Furthermore, there is very little understanding of how different rates of
108 compost addition impact upon the formation of AM (Copetta et al., 2011; Valarini
109 et al., 2009). Taken together, if compost and AM are to both be part of on-farm
110 nutrient management, there is need to develop an understanding of how
111 compost applied at different rates affects the formation and functioning of AM.

112 Whether or not mycorrhizal plants outperform their non-mycorrhizal
113 counterparts, in terms of growth and nutrient acquisition, in systems where
114 composts are used is not well established (Caravaca et al., 2003; Puschel et al.,
115 2008; Roldan et al., 2006). This in part can be explained by the complexities
116 associated with the establishment of appropriate non-mycorrhizal treatments
117 for comparison. Typically, non-mycorrhizal treatments are established by
118 sterilizing the soil to eliminate AMF; however, in doing so other soil biota,
119 including those involved in the decomposition of compost and nutrient
120 mineralization, are also eliminated (Smith and Smith, 1982). While sterilized
121 soils can be back-inoculated with bacterial filtrates after sterilization, this does
122 not completely return all biota to the soil. Another challenge particular to studies
123 using composts, is that even if appropriate non-mycorrhizal treatments are
124 established in which AMF are eliminated and other soil biota are returned to the
125 soil, the compost applied may bring with it propagules of AMF. One solution for
126 overcoming these issues is the use of mycorrhiza defective mutants that do not
127 form AM and their wild-type progenitors that do form AM (Barker et al., 1998;
128 Rillig, 2004; Watts-Williams and Cavagnaro, 2014). This genotypic approach to
129 controlling for the formation of AM overcomes the need to sterilize soils to
130 establish non-mycorrhizal treatments, thereby ensuring that the biological
131 processes responsible for compost decomposition and nutrient mineralization
132 are unaffected (Cavagnaro et al., 2006). This approach is also not compromised
133 where the compost applied to the soil contains propagules of AMF.
134 Results of an experiment which investigated the impact of different rates of
135 compost addition on the formation and functioning of AM are reported here. This
136 study asked two specific questions:

1. Is the addition of compost to the soil, at various rates of application, associated with an increase, a decrease or no change in the formation of AM (measured as percent mycorrhizal colonization of roots)?
2. Where compost is applied to the soil at various rates, does formation of AM enhance plant nutrient acquisition and growth?

To answer these questions a glasshouse experiment was conducted in which a mycorrhiza defective mutant and mycorrhizal wild-type genotype pair of tomato (Barker et al., 1998) were grown separately in soil to which compost was applied at rates of 0, 12.5, 25 and 50 t/Ha. Since AM play an important role in the acquisition of P and Zn, emphasis is placed on plant P and Zn nutrition. The tomato genotypes used in this study have been used extensively in the study of AM in the laboratory and field (e.g. Cavagnaro et al., 2008; Cavagnaro and Martin, 2011; Marschner and Timonen, 2005; Watts-Williams and Cavagnaro, 2012), but not with composts. Importantly, these genotypes are matched in terms of growth in the absence of AMF (Cavagnaro et al., 2004). Thus, this genotypic approach to controlling for the formation of AM provides a unique, and minimally invasive way, of studying AM-compost interactions with the wider soil biota interact.

2 Materials and methods

2.1 Soil, compost and plants

Plastic, free-draining pots (130 mm diameter) were filled with 1 kg of a 20:80 (*W/W*) soil:sand mixture. The field soil, which was collected from Wallenjoe Swamp State Game Park (Lat. = -36.471935, Long. = 144.868512) situated near Rochester in northern Victoria, Australia, is a loam. This soil has a pH of 6.4 ± 0.4, a total C content of 1.9 ± 1.1 %, a total N content of 0.2 ± 0.1 %, and has low concentrations of plant available P (12.8 ± 7.4 mg P/kg soil) and diethylene triamine pentaacetic acid (DTPA)-extractable Zn (1.2 ± 0.7 mg Zn/kg soil). The sand was a coarse washed river sand. This soil:sand mixture, which is referred to as 'soil' hereafter, was used as it further reduces soil nutrient concentrations (plant-available (Colwell) P = 3.8 ± 1.5 mg P/kg soil*;* DTPA-extractable Zn 0.13±0.3 mg Zn/kg soil), has a high AMF inoculum potential, and permits ready and complete extraction of fine roots at the time of harvest (Watts-Williams and Cavagnaro, 2012).

A municipal greenwaste compost was used in this study. Details of the preparation of the compost are given in Ng *et al*. (2014). Key physicochemical properties of the compost (Mean ± S.E.) were: Plant-available (Colwell) P = 1459 ± 30 mg/kg, pH = 8.4 ± 0.1, DPTA-extractable = Zn 82 ± 2 mg Zn/kg, Total C = 16.9 ± 0.3 %, Total N = 1.5 ± 0.003 %, C:N ratio 11.4 ± 0.2. The compost was applied to the soil by placing a layer of compost on the surface of the soil in the pots at rates of 0, 29.9, 59.8 and 119.6 g of compost per pot, which equated to compost additions of 0, 12.5, 25 and 50 t/Ha. Thus, the amounts of P and Zn added to the pots in the compost, in the Low, Medium and High compost addition treatments were 43.6, 87.2 and 174.5 mg P and 2.5, 4.9 and 9.8 mg Zn,

180 respectively. These rates of compost addition are within the range observed
181 under field conditions (Cavagnaro, un-published).
182 In each pot were sown pre-germinated seeds of either a mycorrhiza
183 defective tomato (*Solanum lycopersicum* L.) mutant with reduced mycorrhizal
184 colonization (*rmc*, hereafter), or its mycorrhizal wild-type progenitor *S.*
185 *lycopersicum* cv. 76R (76R, hereafter)(Barker et al., 1998). The seeds were pre-
186 germinated, following surface sterilization with 4% NaOCl, by incubating them
187 on a moist filter paper in a 90 mm diameter Petri Dish in the dark at room
188 temperature. Each treatment was replicated five times, giving a total of 40 pots;
189 we, and others, have found this level of replication to be sufficient in our
190 previous work using this experimental system (see Watts-Williams and
191 Cavagnaro, 2014 for recent review of this work). During the course of the
192 experiment one *rmc* plant in the 25 t/Ha compost addition treatment was lost;
193 there were therefore, only four replicates in this treatment.
194 The plants were grown in a temperature-controlled glasshouse with
195 supplemental lighting (MK-1 Just-a-shade, Ablite Australia), with a 16/8 h
196 day/night photoperiod. Conditions in the glasshouse were: 215±27 μmol/m^2/s^1,
197 and the temperature was maintained at 22°C day and 17°C night. All plants were
198 watered with reverse osmosis (RO) water to 60% of field capacity (by weight),
199 for the duration of the experiment. The plants were arranged randomly in the
200 glasshouse, and were re-randomized on a weekly basis.
201
202 *2.2 Harvesting and analysis*
203 There was one destructive harvest 55 days after planting, as follows. Plants were
204 washed free from their pots with RO water. All of the shoots and a weighed sub-

sample of the roots were oven-dried and shoot dry weights and root dry weights determined. All oven-dried plant material was then ground to a fine powder, and nutrient concentrations determined using radial view inductively coupled plasma–optical emission spectrometry (ICP-OES) (by Waite Analytical Services, http://www.adelaide.edu.au/was/ last accessed June, 2014). A second weighed sub-sample of fresh root material as cleared in KOH (10% W/V) and stained with ink and vinegar, for determination of mycorrhizal colonization using the gridline intersect technique (Giovannetti and Mosse 1980).

2.3 Calculations and data analysis

In an attempt to explore patterns in the data, we calculated a number of mycorrhizal response parameters (following Cavagnaro et al., 2003). The mycorrhizal P response was calculated for both roots and shoots separately, using the individual P content (i.e. µg P/plant) of 76R plants and mean P content of the *rmc* plants (Eq. 1).

$$\%\text{Mycorrhizal P response} = \left(\frac{\text{P content 76R} - \text{mean P content } rmc}{\text{mean P content } rmc}\right) \times 100$$

The mycorrhizal Zn response was calculated similarly for both roots and shoots separately. N.B. a positive mycorrhizal P or Zn response indicates that AM plants contained more P or Zn than their non-mycorrhizal counterparts, whereas negative values indicate that the reverse was true. Mycorrhizal growth responses were also calculated as described in Cavagnaro *et al*. (2003).

All biomass and tissue nutrient concentration data were analyzed by two-way ANOVA – the factors in the analysis were *Genotype* and *Compost Addition Treatment*. Whereas roots of the *rmc* genotype were not colonized by AMF, those

of the 76R genotype were; therefore, colonization for the *rmc* genotype was omitted from the statistical analysis, and the difference in colonization among the compost addition treatments for the 76R genotype were analyzed using one-way ANOVA. Where significant differences were found in the ANOVA's, pairwise comparisons were made using Tukey's HSD. For the mycorrhizal P response and mycorrhizal Zn response data, one-way ANOVA's were performed, again with differences among means identified using Tukey's HSD, where the ANOVA indicated a significant effect of *Compost Addition Treatment*. All data were analyzed using JMP statistical software (version 9.0.0).

3 Results

3.1 Mycorrhizal colonization

Roots of the *rmc* genotype were not colonized by AMF in any of the compost addition treatments, and are therefore not considered further. Addition of compost to the soil at low and intermediate rates did not have a significant impact upon percent colonization of the 76R genotype (Fig. 1, Table 1). In contrast, compost addition at a high rate to 76R plants resulted in a significant reduction in percent colonization compared to where no, or a small amount of compost, was applied (Fig. 1, Table 1).

3.2 Plant growth and nutrition

The shoot dry weight of the *rmc* plants was marginally (~6%) higher than that of the 76R plants, irrespective of compost addition treatment (Fig. 2, Table 1). The root dry weight of both genotypes was matched (Fig. 2). On a whole plant biomass basis (roots plus shoots) the mycorrhizal plants were slightly smaller than the non-mycorrhizal plants (Fig. 2). The addition of compost had a substantial impact on plant growth, with both shoot and rood dry weights increasing significantly, and linearly, with increasing rates of compost application. This may have important implications for considering impacts of compost addition of the formation of AM. Whereas percent colonization of roots by AMF was measured in this study (as in most other studies of AM), colonized root length may be a more appropriate measure of AM fungal abundance in the root. Although root length colonized was not measured in this study, assuming that root length/ dry weight ratio was fairly constant for all treatments it can be concluded that mycorrhizal colonization of the whole root systems (and hence

per plant) actually increased with increasing compost addition, i.e. increasing root biomass (Fig. 2) greatly exceeded decreases in percent colonization where these occurred (Fig. 1).

Acquisition of P and Zn by plants was enhanced by the formation of AM, as reflected in mycorrhizal P and Zn responses calculated on a content basis (Fig. 3a, b, Table 1), and plant nutrient concentrations (Fig. 4a, b, Table 1). Importantly, these patterns in nutrient acquisition differed among compost addition treatments, and between tissue types (roots and shoots). For example, whereas shoot P concentration was higher in the 76R genotype than the *rmc* genotype only where no compost was applied, root P concentrations were higher in the 76R genotype than the *rmc* genotype, irrespective of compost addition treatment. Furthermore, the mycorrhizal P response of the shoots, but not the roots, was strongly positive where no compost was applied. In contrast, the mycorrhizal P response in the roots was greatest at a low rate of compost addition. Generally, the application of compost resulted in an increase in shoot and root P concentrations, but the largest increase was seen at a low rate of compost application. For Zn, there was a benefit of forming AM in terms of Zn acquisition, especially where no compost was added to the soil; this was seen both in terms of nutrient concentrations and mycorrhizal Zn response. The application of compost only resulted in a small increase in Zn concentrations in the shoots of mycorrhizal and non-mycorrhizal plants, and only for the roots of non-mycorrhizal plants, at a low rate of compost application.

4 Discussion

The aim of this study was to assess impact of varying rates of compost addition to the soil, on the formation and functioning of AM. A genotypic approach to controlling for the formation of AM was used to establish mycorrhizal and non-mycorrhizal treatments, with the wider soil biota intact. Results are discussed in the context of the role of AM and compost in the provisioning of nutrients to plants, and their 'compatibility' in the context of biologically regulated nutrient supply systems.

4.1 Compost and the formation of AM

Whereas compost application at low and intermediate rates had little effect of percent AM colonization of roots, the addition of compost to the soil at a high rate (50 t/Ha) resulted in a significant reduction in percent AM colonization of roots (but not colonization per total root biomass, see below also). This reduction in percent colonization of roots by AMF with compost addition has been reported in other studies (e.g. Caravaca et al., 2006), and may be due to release of larger amounts of mineralized P into the soil, which can result in a reduction in percent colonization (Baon et al., 1992). Given the high level of plant-available P in the compost, this is not surprising. Be that as it may, the reductions in colonization reported here were substantially less than those in our earlier work using the same genotypes and soil, where plants were supplied with P (and Zn) in mineral forms (Watts-Williams and Cavagnaro, 2012, 2014). While not measured here, it will be important in future studies to relate this reduction in colonization to rates of P release (i.e. mineralization) from the compost over the course of the growing season of the plants. In support of this,

there was clear visual evidence of the compost having degraded over the course of the experiment, which was likely associated with release of nutrients from the compost. Importantly the relatively small decrease in percent colonization of roots by AMF with increasing compost supply was accompanied by a large increase in root growth. Thus, the decrease in percent colonization, as is measured in most studies on compost and AM (see references cited herein), may actually result in positive effects of compost on the formation of AM being overlooked.

4.2 Compost and AM: plant growth and nutrition

There was little effect of AM on plant growth, with the exception of a slightly smaller growth of 76R plants compared with *rmc*, irrespective of compost addition. Nevertheless, this near matched growth of the *rmc* and 76R genotypes is consistent with most, but not all (see Cavagnaro et al., 2012; Cavagnaro et al., 2010; Cavagnaro et al., 2006; Marschner and Timonen, 2005, for examples) previous studies using these genotypes. In those studies were biomass of the two genotypes was not matched, both positive and negative mycorrhizal growth responses have been observed, and appear to be modulated by environmental and edaphic conditions (Cavagnaro et al., 2012; Cavagnaro et al., 2010; Marschner and Timonen, 2005).

While there was little difference in the growth of the two genotypes (see below also), there was a clear impact of adding compost to the soil, with a substantial (linear) increase in plant growth with increasing compost supply. This increase in growth is likely due to the supply of compost-derived nutrients. While there was clearly an increase in the amount of P taken up by plants with

335 increasing compost supply, it is also likely that the increase in growth with
336 compost supply was due to release of N from the compost. It was not possible to
337 analyze plant tissues for N as well as all other nutrients (due to small sample
338 masses in some treatments); however, this conclusion is supported by the
339 observation of N deficiency symptoms in those plants receiving little or no
340 compost. This is also supported by earlier studies showing that compost can
341 supply plants with substantial amounts of N (e.g. Caravaca et al., 2005;
342 Hargreaves et al., 2008; Mendes Filho et al., 2010). Irrespective of the
343 mechanisms involved, it is clear that compost provided an effective means of
344 enhancing plant growth in this experiment.
345 The formation of AM had a strong positive effect on plant P acquisition,
346 with P concentrations higher in the shoots of the plants that formed AM where
347 no compost was added to the soil. This response, which was also seen in the
348 mycorrhizal P response (based on shoot P content), is consistent with earlier
349 studies where the same genotypes have been grown under low P conditions (see
350 Watts-Williams and Cavagnaro, 2014, for recent review). Interestingly, for roots,
351 whereas there was little difference in root P concentrations and mycorrhizal P
352 response where no composted was added to the soil, there was an increase in
353 root P concentration and mycorrhizal P response where compost was added to
354 the soil. Although soil P levels at the end of the experiment were not measured in
355 this study, this suggests that roots colonized by AMF were better able to access P
356 released from the compost than those not colonized by AMF. These differential
357 responses to compost addition and AM in P concentrations and contents in roots
358 and shoots, highlight the need to consider nutrient allocation *in planta*, as well as
359 total nutrient uptake (Miller et al., 2014). The results also show that it cannot be

concluded simply that lack of positive mycorrhizal responses based solely on biomass indicates that AM have no part in P uptake. For example, it has been shown that when plants form AM plant P uptake via the direct (epidermal) pathway can be reduced and effectively replaced by the indirect AM P uptake pathway (Smith et al., 2004).

As with plant P, the concentration of Zn in the tissues of 76R plants was higher than that of the *rmc* plants where no compost was added to the soil. This was also reflected in positive mycorrhizal Zn responses (on a content basis) for both the roots and shoots of the plants. These results are consistent with our earlier work in which we grew these genotypes in the same soil:sand mixture used here (Watts-Williams and Cavagnaro, 2012). Interestingly, the benefit of forming AM, in terms of Zn acquisition (see mycorrhizal Zn responses), was again most pronounced where rates of compost addition to the soil were lowest, or where no compost was applied. This suggests that some Zn is added to the soil with compost addition, which may in turn render any benefit of forming AM in terms of Zn acquisition less important. It may also suggest that as is the case for P (Smith et al., 2004), when plants are colonized by AMF the direct Zn uptake pathway may become relatively less important than the indirect AM Zn uptake pathway; this; however, is yet to be determined. Irrespective of the mechanisms involved, this is to the author's knowledge, the first study of the combined effects of AM and compost on plant Zn nutrition.

One of the key mechanisms by which AM improve plant nutrient acquisition is via their capacity to grow well beyond rhizosphere depletion zones, gain access to microsites not otherwise accessible to roots, and to rapidly colonize and exploit and nutrient patches (Cavagnaro et al., 2005; Drew et al.,

385 2003; Facelli and Facelli, 2002; Tibbett, 2000). Putting aside the debate as to
386 whether or not AMF can directly access nutrients from organic sources (see
387 Smith and Smith, 2011, for recent discussion), rapid colonization of compost
388 patches, or indeed the soil beneath surface-applied compost, would likely lead to
389 enhanced nutrient capture by AM plants in general. While there is some evidence
390 of increased growth of hyphae of AMF with compost supply (Palenzuela et al.,
391 2002), other studies have reported both an increase and decrease in hyphal
392 growth depending upon plant species (Valarini et al., 2009). Clearly, there is a
393 need for further studies that investigate the impact of compost addition on the
394 extraradical growth of AMF, and ability of AMF to directly colonize compost
395 patches in, or on the surface of, the soil. Furthermore, although P is relatively
396 immobile in the soil (Tinker and Nye, 2000), the formation of AM can reduce the
397 risk of P being lost via leaching (Asghari and Cavagnaro, 2011, 2012; van der
398 Heijden, 2010). Where levels of P in composts added to the soil are high (as was
399 the case with the compost used here), this may be important, especially in a field
400 setting where there is a risk of nutrient leaching. This, along with work on the
401 fate of nutrients added to the soil as compost, should be of high priority.
402
403 *4.3 Conclusions: compost and AM*
404 The aim of this experiment was to assess the dual effects of AM and compost
405 addition on plant growth and nutrition. It was clear in this experiment that
406 compost addition to the soil did not have a deleterious impact on the formation
407 of AM as percent of root length colonized, except at high levels of application, and
408 did not decrease colonization on a whole plant basis. Similarly, the functioning of
409 AM, in this case measured as plant P and Zn uptake, was not adversely affected

by compost addition. Indeed, there was an indication (increased root mycorrhizal P responses) to suggest that the formation of AM better enabled plants to acquire nutrients released from the compost when applied at intermediate to high rates. Whether or not this is due to an effect of AM on mineralization of organic P, for which there is little evidence in the literature, or simply foraging for inorganic P released from the compost, remains to be seen (see Smith and Smith, 2011). The results presented here provide insights into the formation of functioning of AM where nutrients are supplied to the soil in the form of compost. The next step will be to verify these findings in field settings. Taken together, it is concluded that compost and AM have an important role to play in plant nutrient supply. With increasing interest in the use of organic sources of nutrients, especially those in waste materials, in agriculture, this is an important area for further research.

Acknowledgements

This work with supported via the award of a Future Fellowship to the author by the Australian Research Council (FT120100463). The author also wishes to thank Ms Stephanie Watts-Williams for assistance in establishing the experiment. Thanks also to Ms EeLing Ng for providing the greenwaste compost, Prof. Sally Smith, and A. Prof. Susan Barker for access to the tomato genotypes used here, and Prof. Andrew Smith for *very* valuable comments on a draft of the manuscript. Comments from the Editor and two anonymous reviewers also helped to improve the manuscript. Excelsior!

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Figure Legends.

Fig. 1 Mycorrhizal colonization at harvest of 76R plants at four rates of compost addition to the soil. Values are mean ± SE, N= 5. Means followed by the same letter are not significantly different at the P<0.05 level; see also Table 1 for ANOVA results

Fig. 2 Shoot Dry Weight (SDW; above X-axis) and Root Dry Weight (RDW; below x-axis) of 76R (black bars) and *rmc* (white bars) plants at four rates of compost addition to the soil. Values are mean ± SE, N= 5. Means followed by the same letter are not significantly different at the P<0.05 level; see also Table 1 for ANOVA results

Fig. 3 (a) Mycorrhizal P Response (MPR) and **(b)** Mycorrhizal Zn Response (MZnR) of roots (dark gray bars) and shoots (light gray bars) grown at four rates of compost addition to the soil. Values are mean ± SE, N= 5. Means followed by the same letter are not significantly different at the P<0.05 level; see also Table 1 for ANOVA results.

Fig. 4 Concentration of **(a)** P, and **(b)** Zn, in the shoots (above x-axis) and roots (below x-axis) of 76R (black bars) and *rmc* (white bars) plants at four rates of compost addition to the soil. Values are mean ± SE, N= 5. Means followed by the same letter are not significantly different at the P<0.05 level; see also Table 1 for ANOVA results

Table 1 ANOVA Table for mycorrhizal colonization, plant growth and nutrition.

	Genotype	***Compost***	***Genotype* × *Compost***
AM Colonization	N/A	P<0.001	N/A
Shoot Dry Weight (SDW)	<0.05	P<0.0001	ns
Root Dry Weight (RDW)	ns	P<0.0001	ns
Shoot MPR	N/A	P<0.0001	N/A
Root MPR	N/A	P<0.05	N/A
Shoot MZnR	N/A	Ns	N/A
Root MZnR	N/A	P<0.05	N/A
Shoot P concentration	P<0.0001	P<0.0001	P<0.0001
Root P concentration	P<0.0001	P<0.0001	ns
Shoot Zn concentration	P<0.0001	P<0.0001	P<0.05
Root Zn concentration	P<0.0001	P<0.0001	P<0.05

Factors in the analysis were *Genotype*, *Compost* addition treatment and their interaction (*Compost X Genotype*). For AM colonization, Mycorrhizal P Response (MPR) and Mycorrhizal Zn Response (MZnR), *Compost* addition treatment was the only factor in the analysis – see text

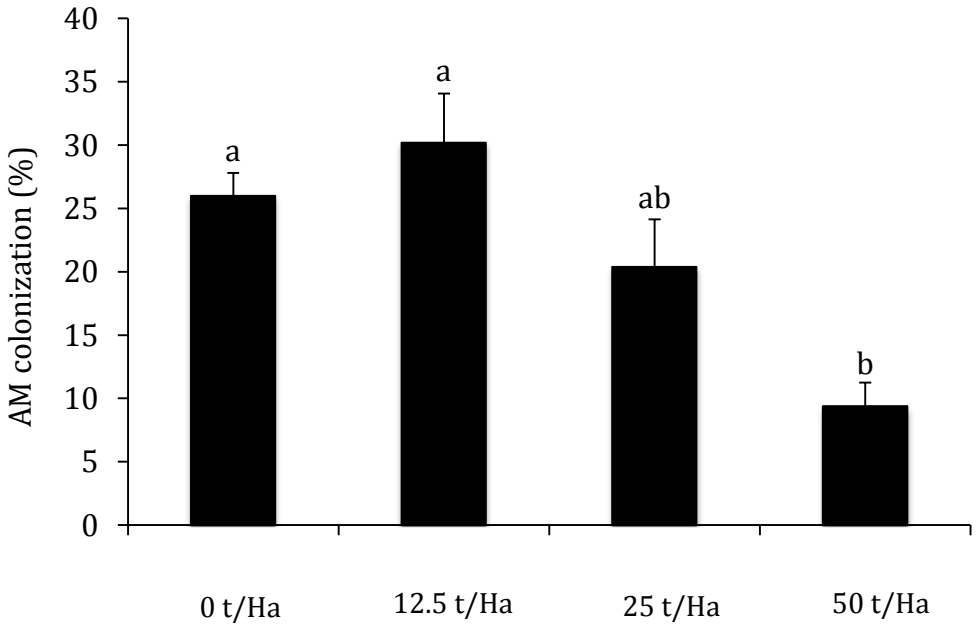
40
35
30
25
20
15
10
5
0
AM colonization (%)
a
a
ab
b
0 t/Ha
12.5 t/Ha
25 t/Ha
50 t/Ha

Figure 1

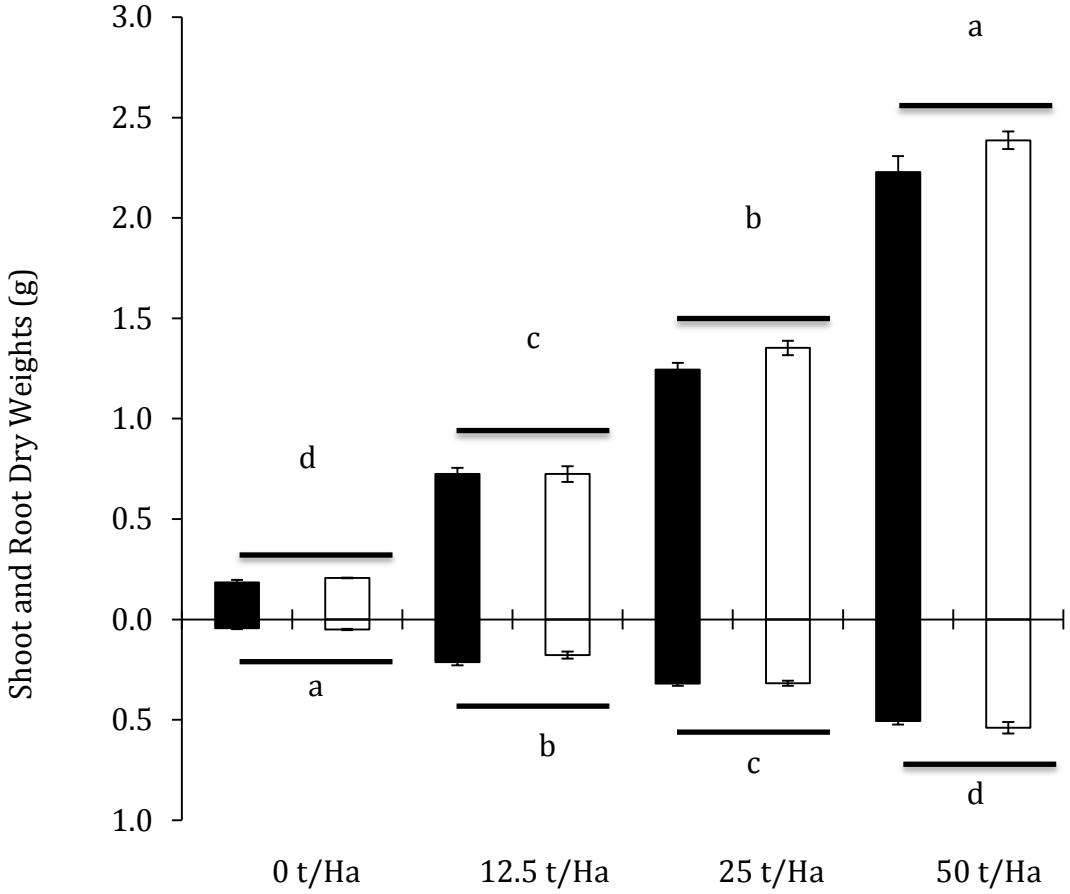
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.5
1.0
Shoot and Root Dry Weights (g)
a
b
c
d
d
c
b
a
0 t/Ha
12.5 t/Ha
25 t/Ha
50 t/Ha

Figure 2

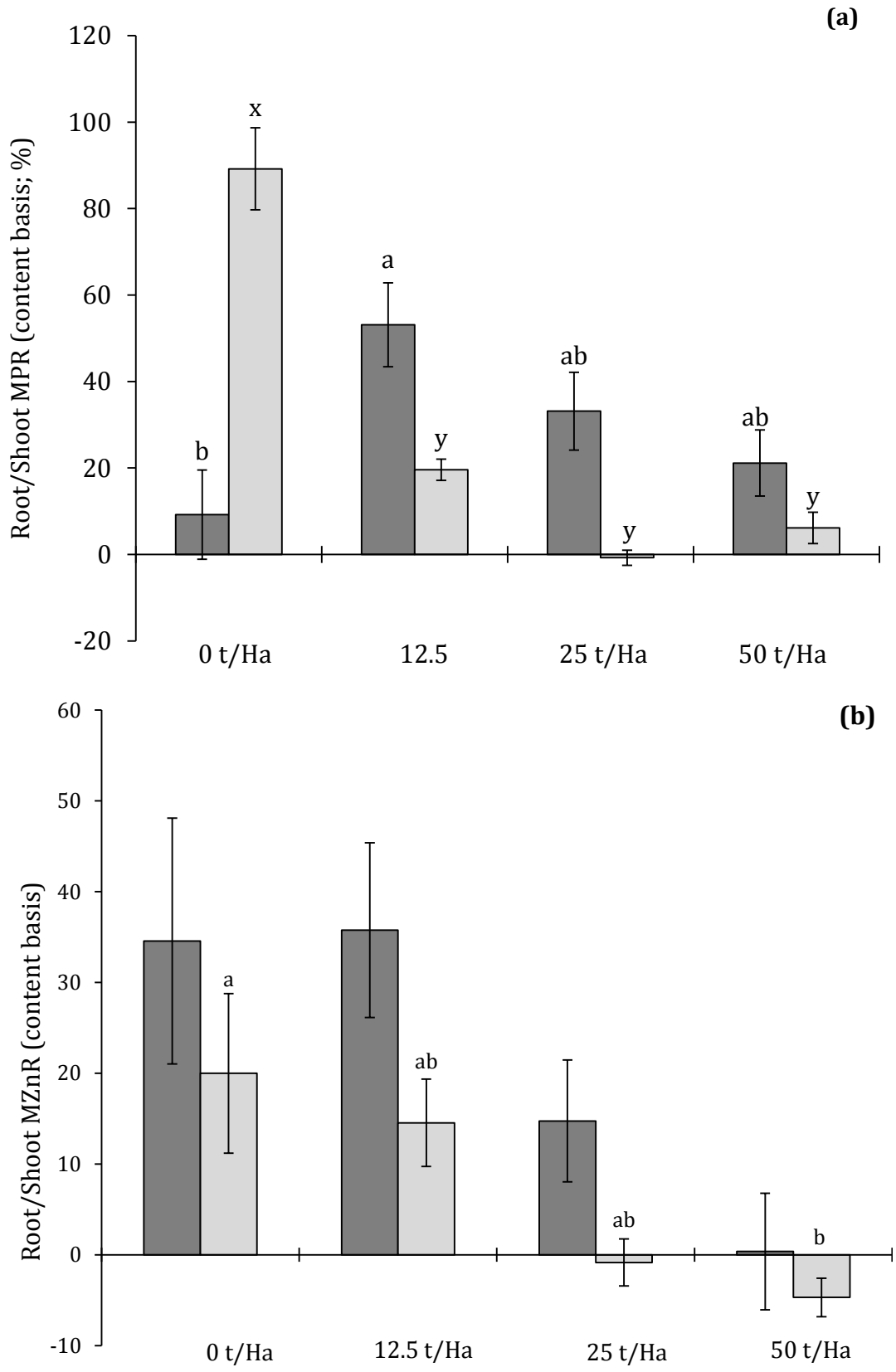
(a)
Root/Shoot MPR (content basis; %)
120
100
80
60
40
20
0
-20
x
a
ab
ab
b
y
y
y
0 t/Ha
12.5
25 t/Ha
50 t/Ha
(b)
Root/Shoot MZnR (content basis)
60
50
40
30
20
10
0
-10
a
ab
ab
b
0 t/Ha
12.5 t/Ha
25 t/Ha
50 t/Ha

Figure 3

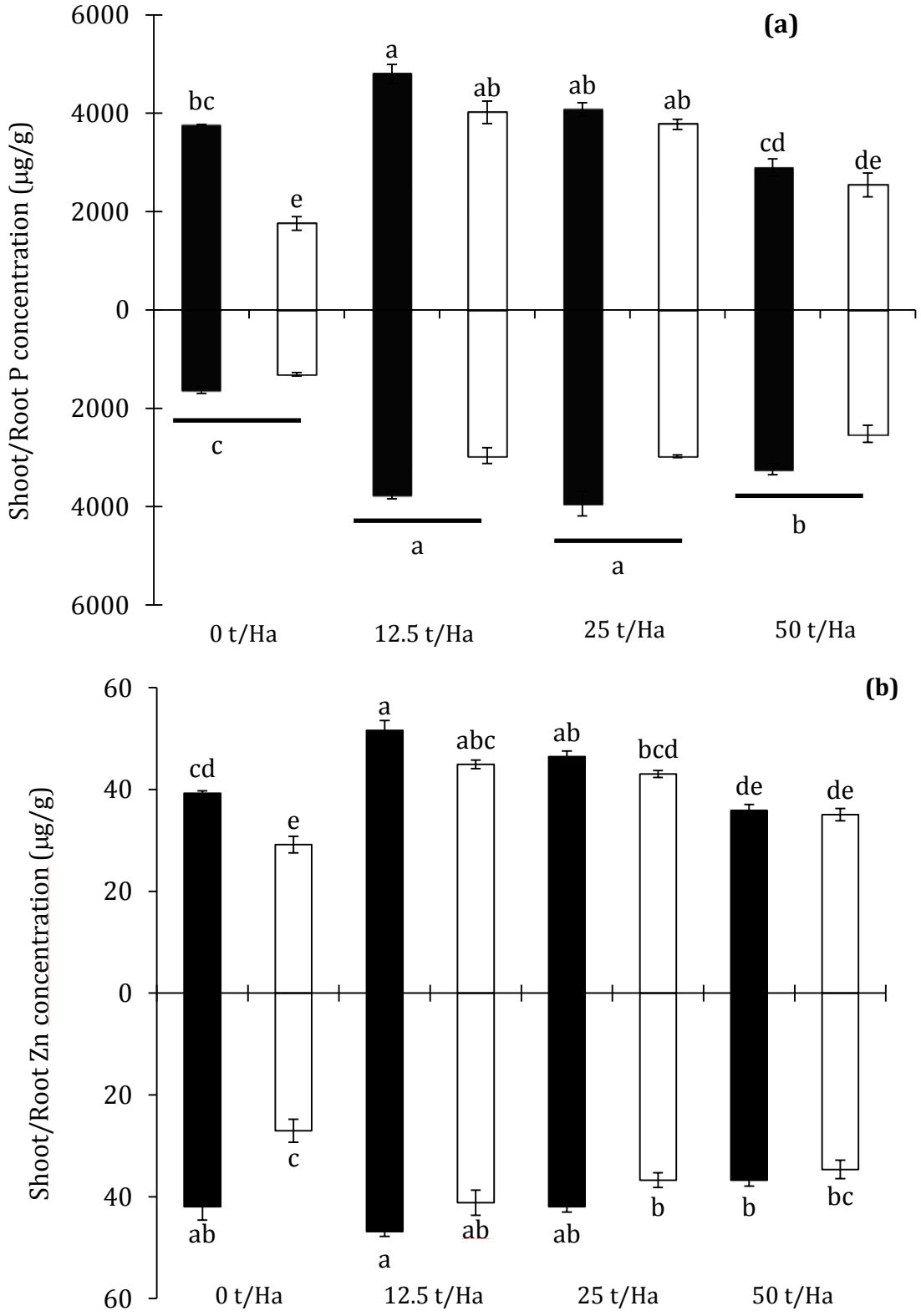
(a)
Shoot/Root P concentration (µg/g)
6000
4000
2000
0
2000
4000
6000
bc
e
a
ab
ab
ab
cd
de
c
a
a
b
0 t/Ha
12.5 t/Ha
25 t/Ha
50 t/Ha
(b)
Shoot/Root Zn concentration (µg/g)
60
40
20
0
20
40
60
cd
e
a
abc
ab
bcd
de
de
ab
c
a
ab
ab
b
b
bc
0 t/Ha
12.5 t/Ha
25 t/Ha
50 t/Ha

Figure 4