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Karen R. Little, Michael T. Rose, William R. Jackson, Timothy R. Cavagnaro, and Antonio F. Patti

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Do lignite-derived organic amendments improve early-stage pasture growth and key soil biological and physicochemical properties?

Karen R Little[A,D], Michael T Rose[B], William R Jackson[A], and Timothy R Cavagnaro[C], Antonio F Patti[A]

[A]School of Chemistry, Monash University, Clayton, VIC 3800, Australia.

[B]NSW Department of Primary Industries, Wollongbar Primary Industries Institute, Wollongbar, NSW 2477, Australia.

[C]School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, PMB1 Glen Osmond, SA, 5064, Australia.

[D]Corresponding author. karen.little@monash.edu

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Abstract

Commercial lignite-derived products, sold mainly as humate preparations, are widely promoted as plant growth stimulants leading to higher crop yields. These products are also claimed to improve key indicators of soil health including soil pH and microbial biomass. In a glasshouse setting, we investigated the effect of six lignite-derived amendments applied at the manufacturer's recommended rate on the early-stage growth of two pasture species, lucerne (*Medicago sativa*) and ryegrass (*Lolium multiflorum* Lam.). We used two soil types common to south-eastern Australia, and following an eight week growing period, assessed soil pH, microbial biomass carbon and mycorrhizal colonization as key indicators of soil health. We hypothesised that

humic acid (HA) and macro-nutrients derived from the products would positively influence pasture growth and soil health indicators. Although significant growth effects were observed in response to some products, the effects were inconsistent across pasture and soil types. Treatment effects on tissue nutrient accumulation were rare, with the exception of increased K in ryegrass in one soil amended with raw brown coal, and decreased N in lucerne in the same soil amended with a granulated slow-release humate product. Further, we did not find any consistent trends in mycorrhizal colonization or microbial biomass carbon in response to individual treatments. Given the variable responses of the plant species and soil types to the amendments used here, we emphasise the need for further mechanistic studies to help understand how these amendments can be used to greatest effect.

Introduction

There is increasing recognition of the need to produce more food on less land, with fewer external inputs (Kremen and Miles, 2012). Much of the increase in food production in recent decades has come from the use of inorganic fertilizers. However, with global fertilizer resources dwindling, and increasing concerns about the energy intensive nature of fertilizer production, there is a need to look to alternative methods to increase agricultural production in a sustainable manner (Tilman et al., 2002). Furthermore, excessive or poorly timed fertilizer application can lead to not only a loss of nutrients from production, but to nutrients being leached into waterways, lost as the potent greenhouse gas N_2O, and a deterioration of soil quality (Fageria, 2010, Chan, 2010, Hoben et al., 2011, Meng et al., 2005). Taken together, there is a need to develop farming systems that maximize nutrient use efficiency.

Healthy soils are the cornerstone of maintaining and enhancing agricultural productivity (Sparling et al., 2006). However, some agricultural practices that are implemented to increase productivity such as increased stocking rates and integrated crop-livestock systems can, if inadequately managed, lead to reduced soil health via soil compaction, and a lowering of fertility and organic matter levels (Hiltbrunner et al., 2012, Houlbrooke, 2011). A loss of organic matter is of particular concern, as it is vital for maintaining the physical structure and stability of soils, as well as providing an energy source for soil microbial communities that drive key soil ecological processes. To help overcome impacts of agricultural intensification on soil health, there has been renewed interest in an agricultural paradigm that places greater reliance on soil organic amendments that improve fertilizer-use efficiency, while increasing levels of soil organic matter (Jackson et al., 2008, Quilty and Cattle, 2011).

Humic substances (HS) are naturally occurring, highly complex, organic mixtures predominantly formed by biochemical reactions that occur during the decay of plant, animal and microbial matter (MacCarthy, 2001). They make up a significant component of soil organic matter and can improve soil properties such as aggregation (Piccolo et al., 1997), water holding capacity, and act as a nutrient 'reservoir' by complexing macro- and micro-nutrients (Imbufe et al., 2005, Alagoz and Yilmaz, 2009, Canarutto et al., 1996, Ferreras et al., 2006, Chen et al., 2004a). The application of HS to soil has been found to stimulate seed germination, and increase the growth and yields of a variety of important agricultural species (Eyheraguibel et al., 2008, Puglisi et al., 2009, Lee and Bartlett, 1976, Nardi et al., 2002, Arancon et al., 2006, Piccolo et al., 1993). However, the effect of adding HS to plants and soils varies with the origin and concentration of the HS applied, and the species of plant and soil type to which it is applied (Rose et al., 2014). Consequently, it is difficult to make generalizations about the mechanisms by which HS impact upon plants and soils. Nevertheless, a number of mechanisms have been suggested including 'hormone-like' effects (Chen et al., 2004a, Muscolo et al., 1998, Muscolo et al., 2012); this however, is the subject of ongoing debate, and there is a need for further detailed studies of a range of HS on more plant species and soil types (Rose et al., 2014).

Lignite (also known as brown coal) is widely used to manufacture a wide range of commercial HS products. Leonardite is often found in association with lignite and is formed by the oxidation of lignite from prolonged exposure to air. Lignite and leonardite are commonly marketed either in 'raw', 'run-of-mine' state or in the form of humic acid (HA) that has been extracted under alkaline conditions (Demirbas et al.,

2006). Leonardite or lignite-derived product (LDP) can be formulated as soluble or slow-release granules and powders, or as liquids that are applied directly to the soil or as a foliar spray (Adani et al., 1998, Çelik et al., 2011, Olk et al., 2013, Seyedbagheri et al., 2012, Verlinden et al., 2010). Products vary in the concentration of HA (generally 25 - 85%) and additional nutrients are commonly incorporated during product formulation. In many instances these products are applied at the manufacturer's recommended rate, with little knowledge of optimal rates, timing and methods of application for a given plant/soil combination. This lack of informed application can lead to sub-optimal outcomes, and highlights the need for direct investigation of the impacts of a range of HS on plants and soils.

Pastures support high value animal-based production systems. Although there is an emerging trend towards the use of HS in the pasture sector in Australia, and beyond, there have been relatively few studies investigating the effect(s) of LDP on the growth of pasture plant species. That said, some insights have been gained; for example, the shoot and root growth of ryegrass has been found to be increased following application of HA derived from manure, compost, decomposed sawdust, straw and peat in both soil and hydroponic-based systems (Asenjo et al., 2000, Bidegain et al., 2000). Similarly, in a field study, there was an increase in the biomass of ryegrass plants following application of commercial LDPs; results however, were variable across soil types (Verlinden et al., 2010). While a number of other studies of impacts of LDP on a range of crop and pasture types have been reported, the majority have been conducted in hydroponic or sand culture experiments rather than soil. Further, although it is often claimed that the addition of HS will improve soil health; there have, however, to our knowledge been no studies of the impacts of HS on common

measures of soil health in pasture soil. For example, the effects of HS on the formation of arbuscular mycorrhizas (AM) has not, to our knowledge, been assessed. However, given that both AM and HS can affect plant growth and nutrition, this is an important knowledge gap. If LDPs are to become a viable strategy for pasture improvement, the recommendations provided to farmers need to be sufficiently robust to return positive results under a wide variety of soil and management conditions.

Here we present results of a glasshouse study in which we sought to determine the effect of six lignite-derived commercial products applied at the manufacturer's recommended rate on pasture based systems. We hypothesised that higher applied rates of product-derived HA and macro-nutrients would result in positive plant growth and soil health effects. This hypothesis was tested by measuring the effect of six LDPs on:

1. the early-stage growth and nutrient contents of ryegrass and lucerne grown in two pasture soils; and
2. soil pH, microbial biomass carbon and mycorrhizal colonization as indicators of soil health.

Materials and methods

LDP characterisation

We assessed six LDPs sourced from three manufacturers; two water soluble solid humate products (A and B), one lignite-mineral blend (C), one granulated slow-release humate product (D), one humate soil conditioner (E) and brown coal sourced directly from the mine (otherwise known as 'run-of-mine' coal) (F) in the Latrobe Valley, Victoria. Key physicochemical properties of the products were quantified as follows: pH was determined in 5 g sub-samples suspended in deionized water (1:5 w/v), using a TPS WP81 meter and probe. An additional 5 g sub-sample was used to determine HA content by repeated alkaline extraction using a modification of the IHSS method, as follows. To each product, 0.1M HCl was added to give a 10:1 acid to LDP ratio (V/W). The slurry was then shaken at 120 rpm for 1 h and allowed to settle for 12 h. The supernatant was removed and discarded. Under an N_2 atmosphere, 0.1M NaOH was added to the solid residue at a ratio of 100:1 (v/w). The slurry pH was adjusted to 12.6 with 1M NaOH and shaken at 120 rpm for 4 h (Hayes et al., 2008). The pH of the slurry was reduced to 9 using 1M HCl, and solids allowed to settle for 12-16 h. The supernatant was removed and retained, and alkaline extraction of the remaining solid repeated a further seven times until the supernatant was a pale brown colour. The supernatants were pooled, and HA precipitated by pH adjustment to 1-2 with 1M HCl. The HA was then dialysed in cellulose membrane dialysis tubing MWCO 12000 (Sigma-Aldrich) in deionised water until the conductivity of the surrounding water was less than 20 uS/m. The HA was then oven dried at 37°C and weighed. This was repeated in triplicate for each LDP. For each of the six products a sample was ground to a fine powder using a mortar and pestle, homogenised and divided into two sub-samples. The first sub-sample was analysed for total C, H and N

157 by dry combustion (by Campbell Microanalytical Laboratory,
158 http://neon.otago.ac.nz/consulting/microlab/, last accessed October 2013). The
159 second sub-sample was analysed for Al, Fe, K, Mn, P, S and Zn by radial view
160 inductively coupled plasma-optical emission spectrometry (by Waite Analytical
161 Services, http://www.adelaide.edu.au/was/ last accessed October 2013). LDP
162 composition is shown in Table 1.
163
164 *Soil collection and characterization*
165 Two soils were used in this study. The first, a Dermosol, was collected from grazed
166 pasture near Stony Creek, Gippsland in south-eastern Victoria (38°35'55"S,
167 146°3'7"E), and the second, a Podosol, from a vegetable farm recently converted from
168 pasture in Cranbourne, Victoria (38°11'6"S, 145°18'50"E). These soils are referred to
169 as Stony Creek (SC) and Cranbourne (CB) soils hereafter. The SC soil was acidic and
170 had a high organic matter content (11.3%), whereas the CB soil was mildly alkaline
171 and had a low organic matter content (2.4%). Both soils were collected in July 2011,
172 from the 0-20 cm soil layer. Immediately following collection, the soils were air dried
173 and sieved to 2 mm. Sub-samples (200 g) of each soil were then analysed for a range
174 of key physicochemical properties (Table 2) (Environmental Analysis Laboratory,
175 Southern Cross University, http://scu.edu.au/eal/, last accessed October 2013). Based
176 on this soil analysis, it was decided to fertilize prior to use in the plant growth
177 experiment; both soils received N, P and K at 100, 40 and 60 kg/ha respectively.
178
179 *Plant growth experiment*

Plastic, free draining pots (16 cm diameter) were filled with 800 g of Stony Creek or 1.1 kg Cranbourne soil. These masses were selected in order to match the field bulk densities for the two soils which were 1.1 and 1.3 g/cm^3 respectively. To each soil the six LDPs were applied separately, following the manufacturer's recommended rate (Table 1). This approach was taken for two reasons. Firstly, this best replicates the decision that is faced by farmers when deciding to, and how to, apply these products. Secondly, the chemical composition of these products was highly variable (see Table 1) and so normalizing application rates to a single property, e.g. % C or nutrient content, would necessitate the application of these products at unrealistic rates in some cases. The LDPs were mixed carefully into the top one cm of soil to simulate soil incorporation during pasture renovation or establishment, by top-dressing prior to smudging, harrowing or aeration as per standard farming practice. The experiment also included a control treatment, in which the soils were not amended with LDP. The pots were then left to equilibrate for three days prior to the sowing of seeds.

To five replicate pots of each treatment, 10 seeds of either lucerne (*Medicago sativa* L.) cv. Aurora or ryegrass (*Lolium multiflorum* Lam.) cv. Bealey were sown to approximately 2 mm below the soil surface. Thus, there were 140 pots in total. Although lucerne is a leguminous plant, N fertiliser was supplied to avoid any interactions between LDPs and rhizobial symbionts. The plants were then transferred to a glasshouse on the Monash University Clayton campus and grown from September–November, 2011. The pots were arranged in a completely randomized design with their position rotated every two days. Conditions in the glasshouse were as follows: light levels maintained with supplemental lighting (16 h day length) averaged 228 ± 14 -µmol m^{-2} s^{-1}, and the temperature was 23.5 ± 1.6°C day and 22.2

205 ± 1.5°C night. Plants were watered to field capacity determined following Agshari and
206 Cavagnaro (2012) with tap water as required, usually every two days. Seed
207 emergence was determined as the number of seeds that emerged within seven days
208 post-seeding and at this time plants were thinned to two per pot.
209
210 *Plant harvesting and analysis*
211 To examine the effects of LDPs on the early stages of growth of these pasture species,
212 there was one destructive harvest eight weeks post-seeding. This approach was taken
213 because to our knowledge, the efficacy of these products at the pasture establishment
214 phase has not been determined. The plants and soil were carefully removed from the
215 pots. The soil was gently shaken from the roots, after which the shoots and roots
216 separated. The roots were then thoroughly washed with water to remove any adhering
217 soil, and rinsed with reverse osmosis (RO) water. The roots were then divided into
218 two sub-samples. The whole shoots and a sub-sample of the roots of each plant were
219 oven dried for three days at 55°C following which shoot dry weight (SDW) and root
220 dry weight (RDW) were determined. The dried plant material was then ground to a
221 fine powder and nutrient concentrations were determined by radial view inductively
222 coupled plasma-optical emission spectrometry (by Waite Analytical Services,
223 http://www.adelaide.edu.au/was/, last accessed October 2013). The second sub-
224 sample of roots was used to assess the percentage of root biomass colonized by
225 mycorrhizae, using gridline intersect method (Giovannetti and Mosse, 1980), after the
226 roots were cleared in KOH (10% w/v) and stained with Trypan blue (omitting phenol
227 from all reagents) (Phillips and Hayman, 1970).
228
229 *Soil analysis*

Soils were refrigerated at -20°C immediately following harvest. As-harvested soils were analyzed for microbial biomass carbon (MBC) by chloroform fumigation (Vance et al., 1987). Sub-samples of each soil (10 g) were fumigated with ethanol-free chloroform for 24 h in a sealed desiccator in the dark. Non-fumigated sub-samples (10 g) were also stored in a dark environment for this period of time. The following day the desiccator was evacuated to remove chloroform from the soils. The fumigated and non-fumigated soils were extracted with 0.5 M K_2SO_4 at a 1:3 (w/v) ratio and filtered. The carbon content of the filtered product was determined by Shimadzu TOC-V CPH/CPN Total Organic Carbon analyser. Soil pH was determined by suspension of an air dried soil sub-sample (5 g) suspended in deionized water (1:5 w/v), using a TPS WP81 meter and probe.

Calculations and data analysis

Due to differences in plant growth between soils, plant biomass, and MBC data were used to calculate plant responses relative to control following (Eq. 1).

$$\% \text{ response} = \left(\frac{mean - mean\ of\ control}{mean\ of\ control}\right) \times 100 \quad (1)$$

Initially, all biomass, tissue nutrient, soil characterisation data were analysed by three -way ANOVA; factors in the analysis were *plant*, *soil type* and *product*. Due to size asymmetry between the two species (i.e. large differences in plant size masking other effects), all data was then re-analyzed by two-way ANOVA with factors in the analysis being *soil type* and *product*. Where significant differences were found,

pairwise comparisons were made using Tukey's honestly significantly different (HSD). To further explore responses to treatments, 95% confidence intervals (CI's) were calculated for plant biomass, mycorrhizal colonization and MBC. These were then used to compare responses relative to zero, with data whose 95% confidence interval being greater or lower than zero considered significantly different. All data were analysed using JMP statistical software (JMP®, Version 10, SAS Institute Inc., Cary, NC).

Results

LDP and soil characterization

The LDPs varied considerably in chemical composition, reflecting differences in the source of lignite, extraction techniques and the addition of nutrients during formulation (Table 1). There were clear differences in the HA content of the products, ranging from 13.9 – 82.3% on a dry weight basis (Table 1). Products A and B contained high levels of K and product C contained higher concentrations of S, P and Ca compared to other products.

The two soils had differing physiochemical properties (Table 2). The Cranbourne soil had low levels of carbon and key plant nutrients. The Stony Creek soil was more acidic with particularly high concentrations of Fe and Mn.

Growth and nutrition

Ryegrass growth and nutrition

The effect of LDP on SDW varied considerably among the treatments and soil types (Fig.1a and Table 3). Analysis by ANOVA indicated a significant interaction

(P=0.04) between soil type and product, with products A, B and F showing inconsistent growth effects in the two soils. Further, in the CB soil, treatment with product A resulted in a significant (as indicated by 95% CI) shoot growth depression , but this was not the case in the SC soil. Products B and D had no significant effect (as indicated by 95% CI) on SDW in CB soil, however caused a negative growth response in SC soil. Overall, there were no strong positive ryegrass shoot growth responses (i.e. as indicated by 95% CI) to any of the LDPs in either soil..

The effect of LDP on RDW varied between the two soils (Fig. 1b) and analysis by ANOVA (Table 3) indicated no significant main effects or interactionsof LDP and soil type. Interestingly, analysis by 95% CI showed that product C gave a significantly positive root growth response in the CB soil while the reverse was true in the SC soil. In addition, taking into account both root and shoot data (Figs. 1a and 1b), product A in the CB soil caused a reduction in SDW, but there was no apparent effect on RDW..

The concentration of K in the shoots of the ryegrass plants was influenced by both LDP and soil type, as indicated by a significant interaction (P=0.005) between these two factors (Table 3). Specifically, in CB soil, product F significantly increased shoot K concentration compared to product E and the control, whereas in SC soil there was no effect. Similarly, for shoot S concentration, there was a significant interaction effect (P=0.02). However, using Tukey's pairwise comparisons, we were not able to identify which treatment means differed significantly; this reflects the more conservative nature of the Tukey's test than the ANOVA. There was a significant main effect of LDP on shoot N concentrations. Product C increased N tissue concentration compared to product D; however, neither differed significantly from the

control. Although there were no further effects of LDP on tissue nutrition, there were significant differences between the effects of each soil on plant nutrient uptake, especially in terms of Mn (higher uptake in SC) and Al (higher in CB) (Tables 3 and 4).

Lucerne growth and nutrition

Similar to ryegrass, the effect of LDP on the SDW of lucerne varied considerably (Fig. 2a). Analysis by ANOVA indicated a significant main effect of soil type, with lucerne shoot growth higher in CB soil compared to SC soil. Products B and C gave a significant growth increase (indicated by 95% CI) in CB soil whereas only product B had a positive effect in SC soil. Similarly, the RDW of lucerne was largely unaffected by LDP addition (Fig. 2b). Only product C caused a positive root growth effect, but this only occurred in CB soil.

With regards to plant nutrition, ANOVA analysis identified a significant interaction ($P=0.05$) between LDP and soil type for shoot N concentration (Tables 3 and 4). Specifically, in CB soil, lucerne treated with product E contained lower concentrations of N compared to the control. For the other nutrients, there was no significant effect on shoot tissue nutrients (Table 4); however, as for ryegrass, soil type did have an effect particularly for Al, Fe and K (higher uptake in CB soil) and Mn (higher in SC soil) (Tables 3 and 4).

Soil biological and physiochemical properties

Ryegrass mycorrhizal colonisation

The effect of LDP on mycorrhizal colonisation was significant, yet variable, between soil types and among products (Fig. 3a). Analysis by ANOVA indicated a significant interaction (P=0.01) between LDP and soil type. Colonization was generally higher in SC soil, however application of product A resulted in higher colonization in CB soil. Comparing products in each soil type, application of product A resulted in a significant increase in colonisation in CB soil compared to products B, C, E and F. In SC soil there was no significant change compared to the control.

Lucerne mycorrhizal colonisation

Analysis by ANOVA indicated main effects of product (P=0.01) and soil type (P<0.0001) on mycorrhizal colonization in lucerne (Table 3). Product A increased mycorrhizal colonization in CB soil but did not in SC soil (Fig. 3b). Interestingly, the application of LDP to SC soil had an overall negative effect on colonization with significant decreases on application of products B, C, D and F.

Microbial biomass carbon

For ryegrass, analysis of MBC by ANOVA indicated a significant interaction (P<0.001) between LDP and soil type. In CB soil, the addition of LDP generally had a positive effect on microbial biomass (Fig. 4a). In particular, products A, B, C, E and F promoted MBC significantly, as verified by 95% CI. In comparison, when LDP was applied to SC there was no significant MBC response. For lucerne, there was no significant main or interactive effects (Table 3) and so regardless of soil type, the application of LDP did not significantly promote or depress MBC (Fig. 4b).

Soil pH

Analysis by ANOVA indicated a significant main effect of soil type but not product on the post-harvest soil pH (Table 3). The pH of CB soil was higher than that of SC soil (Table 5). For both ryegrass and lucerne, the application of LDP to both CB and SC soils did not have a significant effect on soil pH when analysed by ANOVA and 95% CI. **Discussion**

Variable responses in terms of both early-stage pasture growth and measures of soil health to the application of six commercially sourced LDPs applied to two difference soils and pasture species were observed. This finding highlights the need for soil- and plant-specific optimisation when applying these amendments.

Soil and LDP characterization

The considerable differences in chemical composition between LDPs may be attributed to the origin of the parent material, the extraction technique, and additions made during the product formulation process. For example, the comparatively high concentrations of K in products A and B are likely an artefact of the extraction process. In contrast, the relatively elevated P and Ca content of product C reflect the labelling of the product as a humate-fertilizer blend, in which minerals are probably added during the formulation process.

Numerous studies indicate the importance of HS application rate to the plant nutrient availability and the magnitude of the plant growth response (Tahir et al., 2011, Adani et al., 1998, Atiyeh et al., 2002, Tan and Nopamornbodi, 1979, Abayrak and Camas, 2005). In this study, the HA content of the LDPs varied between 13.9 and 82.3% and therefore using the manufacturer's recommended application rate resulted in a wide range of HA actually applied. Investigating the rate of application of HA in isolation is important , and is worthy of further consideration (Rose et al., 2014).

Effect of LDPs on shoot growth

The effect of LDPs on the growth of ryegrass was not consistent between soils, with no observable trends evident on shoot and root dry matter accumulation. Despite products A, B and F having relatively high HA content (50.2, 82.3 and 68.4% respectively), inconsistencies between the growth effects in each soil may suggest that perhaps soil type rather than HA content is an important factor in the performance of these products (Rose et al., 2014).

Product B was the only LDP that consistently improved lucerne shoot growth in both soil types. However, this result was not reflected in the ryegrass, suggesting that this effect may be plant species dependant, as has been seen in other HA studies (Akinremi et al., 2000, Lodhi, 2013). Product B is a soluble humate, recommended by the manufacturer to be applied as a liquid, and so for this plant type, may have been more readily accessible. Similarly, Verlinden et al. (2009) reported higher shoot yield of permanent grassland in field trials, and Italian ryegrass in pot trials with a liquid humate product compared to the solid form. Thus mode of application may be an important consideration.

Effect of LDPs on root growth

With respect to root growth, treatment of both lucerne and ryegrass in the sandy CB soil with product C increased biomass by 28 and 45%, respectively. Although this product is a mineral blend high in P and Ca, leaf tissue nutrient analysis indicated that these elements were not elevated, and so this effect is unlikely due to increased

uptake. Interestingly, this same product reduced the root growth of both pasture types in SC soil indicating a soil dependent effect. Previous studies indicate variable shoot and root growth effects in soils with differing organic matter content, with more pronounced growth effects in low organic matter soils (Fagbenro and Agboola, 1993, Kunkel and Holstad, 1968, Lee and Bartlett, 1976). The effects observed in this study may therefore be related to the difference in organic matter levels between the two soils, but with differences also in pH, texture and nutrient content. The precise mechanisms for root stimulation/inhibition remain unknown.

Effect of LDPs on ryegrass and lucerne nutrition

Previous studies have shown varied responses in the uptake of macro- and micro-nutrients which could be related to the crop type, growing media or source of HA (Adani et al., 1998, Verlinden et al., 2009, Verlinden et al., 2010, Akinremi et al., 2000, Fagbenro and Agboola, 1993, Ascaso et al., 1985, Tan and Nopamornbodi, 1979). Despite differences in chemical composition and application rates of LDPs, the only differences identified in nutrient uptake for this study were in K and N.

Significant increase in shoot tissue K was seen in ryegrass in CB soil with product F. Other studies have shown increases in K in a range of crop types (Verlinden et al., 2009, Yolcu et al., 2011, Tahir et al., 2011) while others have shown no change (Tan and Nopamornbodi, 1979, Pilanal and Kaplan, 2003, Liu et al., 1998). Product F did not have a high level of inherent K, so this was not the source. Despite elevated K in products A and B, no tissue accumulation of K was detected in ryegrass or lucerne treated with these products. This may be due to the low application rate or the availability of product-derived K for plant utilisation.

LDP application to both ryegrass and lucerne had an effect on shoot N concentration. In ryegrass, accumulation of N detected in ryegrass treated with product C was significantly higher compared to that treated by product D. Product D is a slow-release product and may not have had time to elicit an effect in the short growing period. In lucerne a decrease in tissue N resulted from application of product E to CB soil compared to the control, yet this was not accompanied by a growth reduction. Such a result is in agreement with Akinremi et al. (2000), who found decreased N uptake in canola grown in soil amended with milled leonardite. There are also studies with contrasting results, showing the application of HA to increase tissue N concentration in a range of crops (Verlinden et al., 2009, Verlinden et al., 2010, Çimrin et al., 2001) or show increase or decrease depending on the applied rate (Tan and Nopamornbodi, 1979). As has been demonstrated for plant growth (Rose et al., 2014), nutritional effects may also be dependant on the origin and rate of HA application, crop and/or soil type. It is clear that more investigation into the effect of HA on nutrient cycling is required.

Indicators of soil health

Due to their complex chemical nature and high C-content, HA containing LDPs are likely to directly and indirectly interact with soil microorganisms. Research to date on these interactions is limited, despite extensive knowledge about the general role of soil microorganisms in plant health. The formation of arbuscular mycorrhizabetween a specialized group of soil fungi and most plant species enables enhanced uptake of plant essential nutrients which can improve plant growth (Smith and Read, 2010) . It

has been hypothesized that the presence of HA influences nutrient availability for plant uptake (Chen et al., 2004b).

Product A had a stimulatory effect on mycorrhizal colonisation of ryegrass in both soils, and lucerne in CB. While studies of the effect of humic acid on mycorrhizal colonisation are limited, a similar stimulatory effect was seen by Gryndler *et al*. (2005) in maize roots. For lucerne, products B, C, D and F had a depressive effect on colonization in SB soil. Vallini *et al*. (1993) also saw a similar effect; however, it was at a HA concentration of 3000 mg/kg which is well in excess of the concentrations used here. Levels of colonisation were similar to those previously reported for pasture (Ryan and Ash, 1999), with a lower percentage of roots colonized in the ryegrass which may be due to its fine, branched root structure, more easily able to access nutrients (Schweiger et al., 1995).

Interestingly, with the exception of product D, MBC was increased by LDPs in ryegrass in CB soil with no significant response in lucerne, or either plant type in SC soil. Microbial biomass carbon is a sensitive measure to monitor changes in soil organic matter status (Sparling, 1992); hence, the lack of an effect of the LDPs on MBC in the high-organic matter SC soil is not unexpected in a soil which already has high levels of HS. There have been limited studies that investigate the effect of HA on the soil microbial community although it has been found in a nutrient culture medium that the structure of the humic product plays a role in promotion or suppression of populations within the soil microbial community (Visser, 1985).

Conclusions

The application of a range of commercially-available LDPs to lucerne or ryegrass in two contrasting soils gave variable results in terms of plant growth and soil health measures. There was not a clear, consistent link between HA and nutrient content of the products, and positive plant growth and soil health indicator effects. In agreement with others,it is possible that application rates were too low to elicit a significant agronomic response (Feibert et al., 2003, Hartz, 2010, Duval et al., 1998, Chen et al., 2004b), Further investigation is needed into the mechanistic interaction between the LDP and the plant, and the impact on nutrient cycling. This along with studies that include a wide range of application rates, and longer term glasshouse and field studies may enable the matching of each LDP with specific soil and plant types in specific environmental settings.

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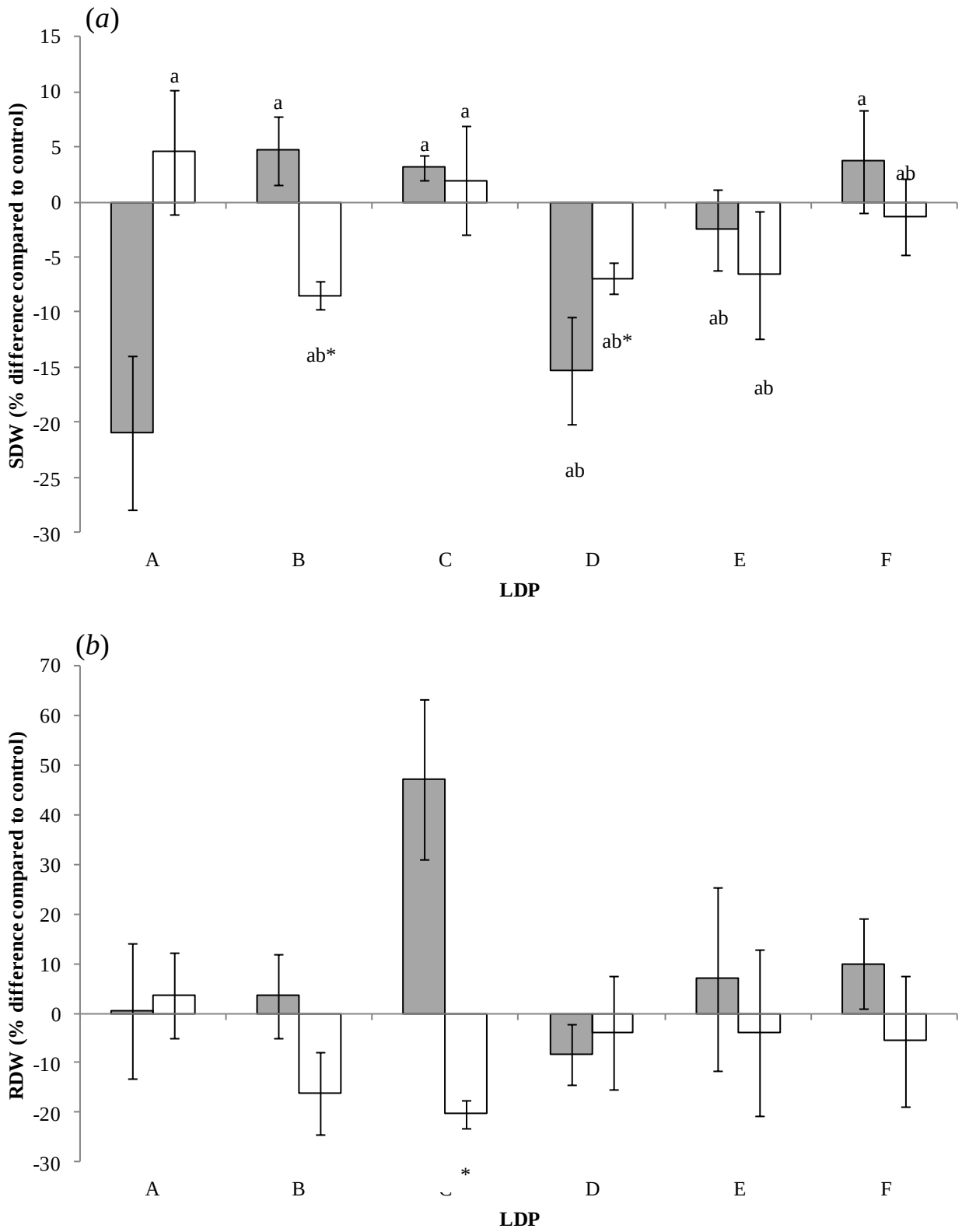

Fig. 1a Dry weight of shoots (SDW) (a) and roots (RDW) (b) of ryegrass grown for 56 days in soils amended with lignite-derived products (LDP). Letters denoting X-axis categories refer to individual LDPs as indicated in Table 1. Values are percentage (%) change compared to the control value. Actual data is included in the Supplementary information. Grey bars represent Cranbourne soil, white bars Stony Creek soil. Values allocated the same letter were not significantly different at the $P<0.05$ level as assessed by Tukey's HSD. * indicates a significant difference from zero as assessed by 95% confidence interval.

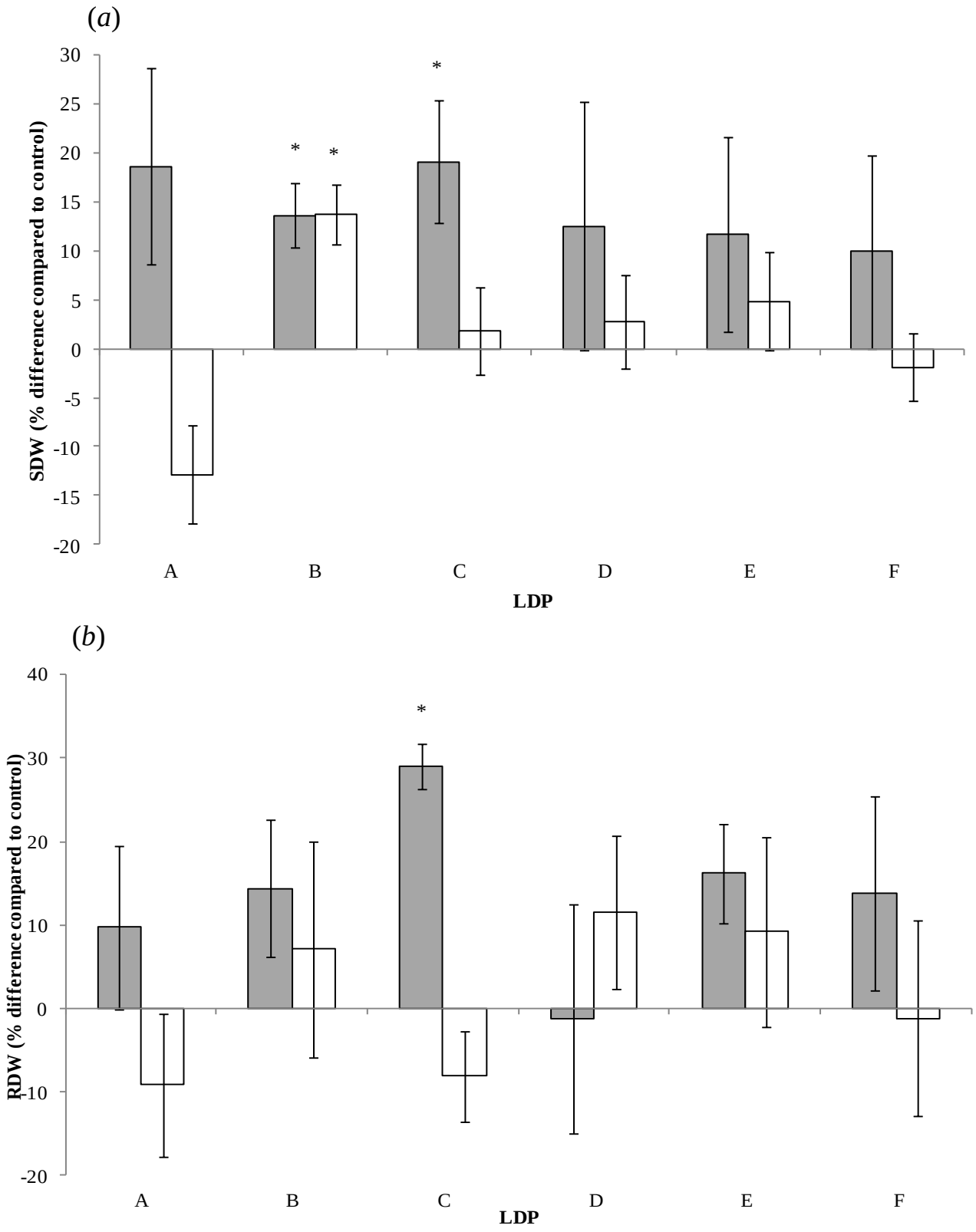

Fig. 2a Dry weight of shoots (SDW) (a) and roots (RDW) (b) of lucerne grown for 56 days in soils amended with lignite-derived products (LDP). Letters denoting x-axis categories refer to individual LDPs as indicated in Table 1. Values are percentage (%) change compared to the control value. Actual data is included in the

(*a*)

Supplementary information. Grey bars represent Cranbourne soil, white bars Stony Creek soil. No significant difference was detected at the P<0.05 level as assessed by Tukey's HSD. * indicates a significant difference from zero as assessed by 95% confidence interval.

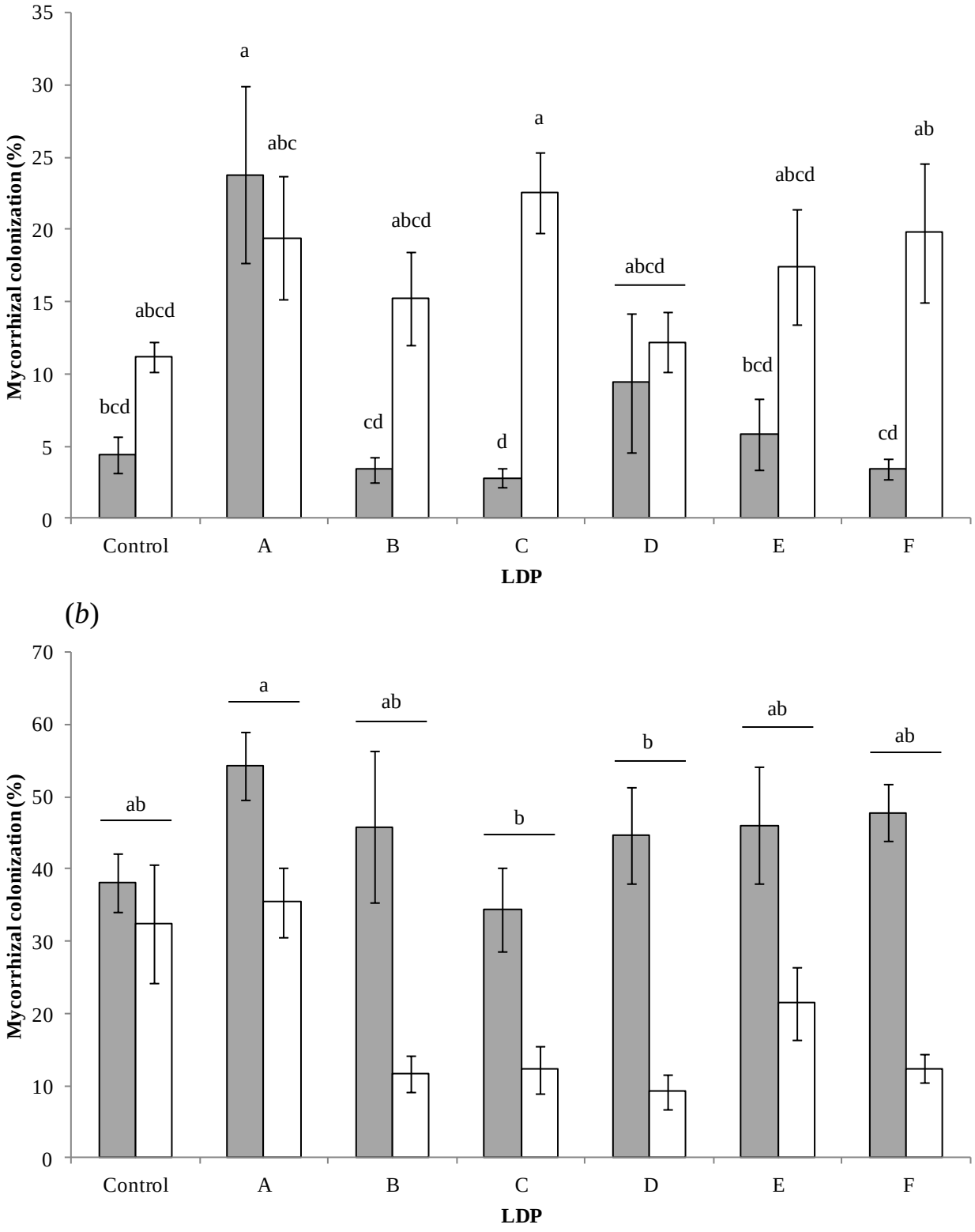

Fig. 3a Mycorrhizal colonization in roots of ryegrass (a) and lucerne (b) grown for 56 days in soils amended with lignite-derived products (LDP). Letters denoting x-axis categories refer to individual LDPs as indicated in

Table 1. Values followed by the same letter were not significantly different at the $P<0.05$ level as assessed by Tukey's HSD. In the ANOVA analysis there was a significant main effect of soil type irrespective of LDP application: see text for results. There was also a significant main effect of LDP application irrespective of soil type; these differences are indicated by letters above bars connected with a horizontal line to indicate where soil types should be pooled over LPD treatment. Grey bars represent Cranbourne soil, white bars Stony Creek soil.

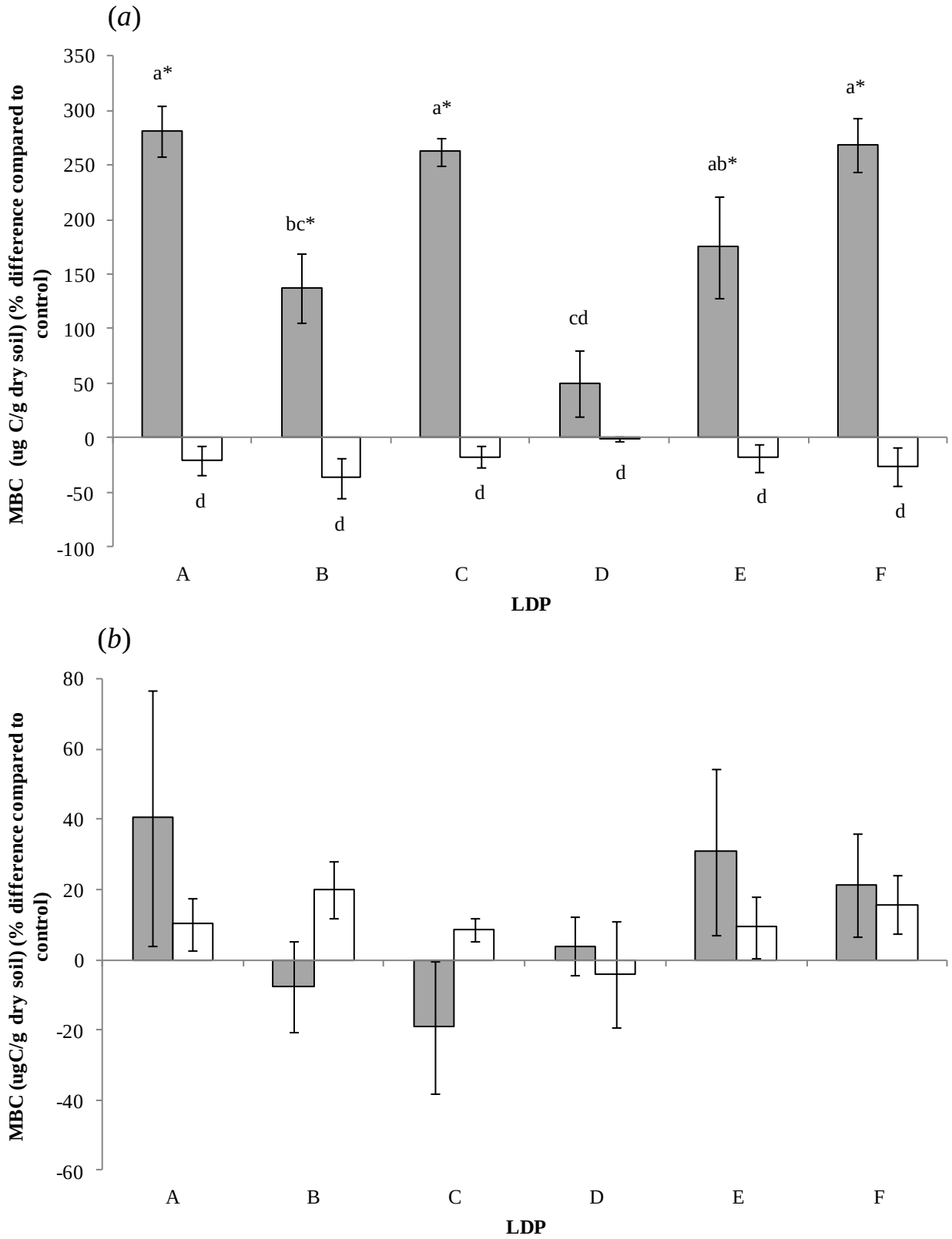

Figure 4a Microbial biomass carbon (MBC) associated with ryegrass (a) and lucerne (b) grown for 56 days in soils amended with lignite-derived products (LDP).in soil. Letters denoting x-axis categories refer to individual LDPs as indicated in Table 1. Values are percentage (%) change compared to the control value. Actual data is included in the Supplementary information. Grey bars represent Cranbourne soil, white bars Stony Creek soil.

Values allocated the same letter were not significantly different at the P<0.05 level as assessed by Tukey's HSD. * indicates a significant difference from zero as assessed by 95% confidence interval.

Table 1 Description and composition of lignite-derived products (LDPs)

	Application rate *	pH	% moisture	% HA	% C	% H	% N	% S	% P	% K	% Na	% Mg	% Ca	% Fe	% Al
Soluble humate granules (A)	4 kg/ha	9.4	4.5	50.2	47.5	4.19	1.32	0.33	0.02	8.50	2.70	0.14	0.95	1.04	1.00
Soluble humate powder (B)	10 L/ha	10.4	9.1	82.3	42.1	4.45	0.73	0.23	0.001	16.90	0.26	0.20	0.10	0.23	2.90
Organic-mineral blend (C)	1 t/ha	5.2	3.8	13.9	26.0	2.74	0.98	1.70	5.30	0.23	0.59	0.84	15.7	1.65	1.09
Slow release granules (D)	50 kg /ha	4.2	6.2	75.3	56.5	3.5	1.21	0.4	0.02	0.07	1.64	0.07	0.30	0.35	0.61
Humate soil conditioner (E)	1 t/ha	4.8	39.5	26.1	56.1	4.95	0.55	0.61	0.10	0.08	0.20	0.31	1.29	0.71	0.56
ROM lignite coal (F)**	5 t/ha	4.5	51.9	68.4	66.4	4.84	0.66	0.21	0.0001	0.02	0.07	0.24	0.49	0.20	0.01

* As recommended by the LDP manufacturer

** No recommended application rate provided. Rate was determined by an estimate of economic viability

Table 2 Physicochemical properties of the soils before the addition of lignite-derived products (LDPs)

	Cranbourne	Stony Creek
pH (water)	8.0	5.2
OM (%)	2.4	11.3
C (%)	1.4	6.5
N (%)	0.1	0.5
Ca (mg/kg)	1691	824
Fe (mg/kg)	104	528
K (mg/kg)	157	87
Mn (mg/kg)	4	37
P (Cowell) (mg/kg)	86	49
S (mg/kg)	52	20
Zn (mg/kg)	3.5	7
Texture	Sandy	Loam

Table 3 ANOVA summary table for all response variables. Factors in the analysis were *S* soil and *L* LDP addition treatment. Both main effects and interaction terms are indicated. **P*<0.05; ***P*<0.01; ****P*<0.0001

	Ryegrass	Lucerne

	S	L	S x L	S	L	S x L
Shoot dry weight	***	*	**	***	ns	ns
Root dry weight	ns	ns	ns	ns	ns	ns
Shoot Al concentration	**	ns	ns	**	ns	ns
Shoot Ca concentration	ns	ns	ns	**	ns	ns
Shoot Fe concentration	ns	ns	ns	***	ns	ns
Shoot K concentration	***	ns	*	***	ns	ns
Shoot Mg concentration	***	ns	ns	**	ns	ns
Shoot Mn concentration	***	ns	ns	***	ns	ns
Shoot P concentration	***	ns	ns	***	ns	ns
Shoot S concentration	***	ns	*	*	ns	ns
Shoot Zn concentration	***	ns	ns	***	ns	ns
Shoot N (%)	*	*	ns	**	*	*
Shoot C (%)	ns	ns	ns	*	ns	ns
Mycorrhizal colonization	***	***	*	***	*	ns
Microbial biomass C	***	ns	ns	ns	ns	ns
Soil pH	***	ns	ns	***	ns	ns

Table 4 Nutrient composition of ryegrass and lucerne shoot tissue harvested at 56 days post-seeding, grown in Cranbourne (CB) and Stony Creek (SC) soils amended with lignite-derived products (LDPs). Mean values are indicated (n=5) and values in parentheses are +/- SE. Values allocated the same letter were not significantly different at the $P<0.05$ level as assessed by Tukey's HSD

Plant	Soil	LDP	Al mg kg^{-1}	Fe mg kg^{-1}	K g kg^{-1}	Mn mg kg^{-1}	P g kg^{-1}	S g kg^{-1}	Zn mg kg^{-1}	%C	%N
Ryegrass	Cranbourne	A	90(26)	90(19)	21.8(0.7)^{ab}	26(2)	3.0(0.2)	1.9(0.1)	38(3)	40.4(0.4)	0.7(0.1)
		B	49(10)	65(6)	22.6(1.4)^{ab}	23(1)	2.6(0.1)	2.7(0.2)	37(1)	40.7(1.0)	0.9(0.1)
		C	69(35)	84(20)	24.0(1.3)^{ab}	24(2)	2.9(0.1)	2.3(0.2)	38(2)	39.8(0.7)	1.0(0.1)
		D	54(15)	61(8)	21.3(1.5)^{ab}	24(3)	2.8(0.1)	1.9(0.2)	32(2)	39.6(0.5)	0.6(0.1)
		E	44(18)	65(9)	19.8(0.7)^{b}	23(3)	2.9(0.1)	2.1(0.2)	38(3)	40.7(1.1)	0.8(0.1)
		F	37(4)	58(4)	25.6(1.2)^{a}	20(2)	3.1(0.1)	2.7(0.3)	36 (2)	40.5(0.5)	0.9(0.1)
		Control	69(33)	79(19)	21.2(0.4)^{b}	24(2)	2.8(0.2)	2.4(0.2)	36(3)	39.6(0.8)	0.8(0.1)
	Stony Creek	A	26(13)	69(21)	7.9 (0.3)^{c}	149(9)	2.5(0.1)	2.2(0.1)	56(4)	39.7(0.9)	0.9(0.1)
		B	22(8)	66(9)	7.3(0.3)^{c}	148(7)	2.4(0.1)	1.8(0.1)	59(4)	40.9(0.3)	0.8(0.1)
		C	31(20)	75(25)	7.9(0.6)^{c}	131(13)	2.6(0.1)	1.9(0.1)	55(2)	40.8(0.7)	1.0(0.1)
		D	26(12)	74(21)	6.9(0.4)^{c}	155(8)	2.2(0.1)	1.8(0.2)	53(5)	41.8(0.9)	0.8(0.1)
		E	16(5)	68(15)	7.3(0.5)^{c}	145(11)	2.2(0.1)	1.9(0.1)	52(5)	41.3(0.7)	0.8(0.1)
		F	23(11)	67(12)	6.3(0.6)^{c}	152(11)	2.1(0.1)	1.9(0.1)	56(2)	40.1(1.1)	0.9(0.1)
		Control	14(2)	68(9)	8.6(0.7)^{c}	152(5)	2.6(0.1)	2.0(0.1)	63(5)	40.3(0.2)	1.1(0.1)
Lucerne	Cranbourne	A	56(27)	76(17)	17.5(1.0)	20(2)	3.1(0.1)	2.2(0.1)	27(2)	39.2(0.6)	1.3(0.1)^{abc}
		B	33(6)	79(8)	18.4(0.8)	20(1)	2.8(0.1)	2.1(0.2)	26(1)	40.8(0.7)	1.4(0.1)^{abc}
		C	84(52)	172(105)	17.0(0.5)	21(1)	3.0(0.1)	1.9(0.1)	25(2)	39.5(0.9)	1.4(0.1)^{abc}
		D	58(21)	103(19)	16.9(0.9)	24(3)	2.9(0.9)	2.4(0.2)	26(2)	40.0(0.6)	1.2(0.1)^{bc}
		E	53(10)	88(9)	17.1(1.3)	21(2)	2.9(0.2)	2.2(0.1)	26(1)	40.9(0.5)	1.2(0.1)^{c}
		F	45(17)	101(21)	17.3(0.7)	20(2)	3.1(0.1)	2.2(0.2)	27(1)	40.5(0.9)	1.5(0.1)^{abc}
		Control	41(11)	93(14)	17.9(0.6)	23(2)	2.9(0.6)	2.7(0.4)	27(1)	41.2(0.8)	1.8(0.1)^{a}
	Stony Creek	A	11(1)	49(3)	9.8(0.8)	124(8)	2.2(0.1)	1.8(0.1)	34(4)	41.1(0.8)	1.4(0.1)^{abc}
		B	17(5)	54(8)	8.6(0.4)	150(19)	2.1(0.2)	2.1(0.2)	31(4)	41.5(0.6)	1.4(0.1)^{abc}
		C	9(1)	47(2)	10.6(0.6)	155(5)	2.5(0.2)	2.1(0.2)	35(3)	42.3(1.3)	1.6(0.1)^{abc}
		D	18(3)	59(11)	8.8(0.3)	139(12)	1.9(0.2)	2.0(0.1)	32(3)	40.5(0.3)	1.5(0.1)^{abc}
		E	11(1)	55(6)	8.9(0.4)	163(14)	2.0(0.2)	2.0(0.1)	37(3)	41.1(0.2)	1.6(0.1)^{abc}
		F	15(3)	59(9)	9.8(0.4)	177(22)	2.3(0.1)	2.0(0.1)	36(3)	41.0(0.4)	1.7(0.1)^{ab}
		Control	15(2)	52(3)	9.2(0.4)	144(11)	2.0(0.1)	1.9(0.2)	33(3)	41.4(0.3)	1.5(0.1)^{abc}

Table 5 Post harvest pH of soils following 56 days of ryegrass or lucerne growth. LDP refers to lignite-derived product, as indicated in Table 1. Values in parenthesis are +/- SE.

Plant	Soil	LDP	Soil pH ryegrass	Soil pH lucerne
Ryegrass	Cranbourne	A	7.57(0.03)	7.49(0.07)
		B	7.69(0.04)	7.67(0.03)
		C	7.62(0.04)	7.59(0.01)
		D	7.58(0.05)	7.56(0.05)

		E	7.61(0.04)	7.64(0.03)
		F	7.52(0.03)	7.58(0.04)
		Control	7.60(0.05)	7.57(0.04)
	Stony Creek	A	4.75(0.02)	4.66(0.08)
		B	4.69(0.02)	4.63(0.07)
		C	4.73(0.03)	4.72(0.02)
		D	4.67(0.02)	4.58(0.04)
		E	4.70(0.05)	4.69(0.02)
		F	4.65(0.02)	4.61(0.03)
		Control	4.64(0.03)	4.60(0.03)
Lucerne	Cranbourne	A		
		B		
		C		
		D		
		E		
		F		
		Control		
	Stony Creek	A		
		B		
		C		
		D		
		E		
		F		
		Control		

Supplementary table. Mean shoot (SDW) and root dry weight (RDW), and microbial biomass carbon (MBC) following 56 days of ryegrass or lucerne growth. LDP refers to lignite-derived product as indicated in Table 1. Values in parenthesis are +/- SE.

Soil	LDP	SDW ryegrass (g)	SDW lucerne (g)	RDW ryegrass (g)	RDW lucerne (g)	MBC ryegrass (ug C/g dry soil)	MBC lucerne (ug C/g dry soil)
Cranbourne	A	5.89(0.52)	6.33(0.53)	3.15(0.43)	2.44(0.22)	0.12(0.01)	0.15(0.04)
	B	7.79(0.23)	6.06(0.17)	3.24(0.27)	2.54(0.18)	0.08(0.01)	0.10(0.01)
	C	7.68(0.09)	6.35(0.33)	4.61(0.51)	2.87(0.06)	0.12(0.00)	0.08(0.02)
	D	6.30(0.36)	6.01(0.68)	2.87(0.19)	2.20(0.30)	0.05(0.01)	0.11(0.01)
	E	7.26(0.27)	5.96(0.53)	3.35(0.58)	2.58(0.13)	0.09(0.01)	0.14(0.02)
	F	7.72(0.35)	5.86(0.52)	3.44(0.29)	2.53(0.26)	0.12(0.01)	0.13(0.02)
	Control	7.44(0.33)	5.34(0.57)	3.13(0.37)	2.22(0.23)	0.03(0.01)	0.10(0.01)
Stony Creek	A	9.62(0.52)	6.36(0.36)	3.80(0.32)	2.21(0.21)	0.47(0.08)	0.71(0.05)

B	8.43(0.12)	8.30(0.22)	3.08(0.31)	2.61(0.32)	0.37(0.11)	0.78(0.05)
C	9.39(0.46)	7.43(0.33)	2.93(0.10)	2.23(0.13)	0.49(0.06)	0.70(0.02)
D	8.57(0.13)	7.50(0.35)	3.53(0.42)	2.72(0.22)	0.58(0.01)	0.62(0.10)
E	8.60(0.53)	7.65(0.37)	3.53(0.62)	2.66(0.28)	0.48(0.08)	0.71(0.06)
F	9.08(0.32)	7.16(0.26)	3.47(0.48)	2.41(0.29)	0.43(0.11)	0.75(0.05)
Control	9.20(0.38)	7.30(0.36)	3.67(0.20)	2.44(0.22)	0.59(0.03)	0.65(0.09)