Plant Soil (2015) 395:7–20
DOI 10.1007/s11104-015-2427-3

REGULAR ARTICLE

Enhanced biological N_2 fixation and yield of faba bean (*Vicia faba* L.) in an acid soil following biochar addition: dissection of causal mechanisms

Lukas Van Zwieten · Terry Rose · David Herridge · Stephen Kimber · Josh Rust · Annette Cowie · Stephen Morris

Received: 17 December 2014 / Accepted: 20 February 2015 / Published online: 12 March 2015

Abstract
Background and aims Acid soils constrain legume growth and biochars have been shown to address these constraints and enhance biological N_2 fixation in glasshouse studies. A dissection of causal mechanisms from multiple crop field studies is lacking.
Methods In a sub-tropical field study, faba bean (*Vicia faba* L.) was cultivated in rotation with corn (*Zea mays*) following amendment of two contrasting biochars, compost and lime in a rhodic ferralsol. Key soil parameters and plant nutrient uptake were investigated alongside stable ^{15}N isotope methodologies to elucidate the causal mechanisms for enhanced biological N_2 fixation and crop productivity.
Results Biological N_2 fixation was associated with plant Mo uptake, which was driven by reductions in soil acidity following lime and papermill (PM) biochar amendment. In contrast, crop yield was associated with plant P and B uptake, and amelioration of soil pH constraints. These were most effectively ameliorated by PM biochar as it addressed both pH constraints and low soil nutrient status.
Conclusions While liming resulted in the highest biological N_2 fixation, biochars provided greater benefits to

Responsible Editor: Johannes Lehmann.

Electronic supplementary material The online version of this article (doi:10.1007/s11104-015-2427-3) contains supplementary material, which is available to authorized users.

L. Van Zwieten · S. Kimber · J. Rust · S. Morris
NSW Department of Primary Industries, Bruxner Highway, Wollongbar, NSW, Australia

S. Kimber
e-mail: stephen.kimber@dpi.nsw.gov.au

J. Rust
e-mail: joshua.rust@dpi.nsw.gov.au

S. Morris
e-mail: stephen.morris@dpi.nsw.gov.au

L. Van Zwieten · T. Rose
Southern Cross Plant Science, Southern Cross University, Lismore, NSW, Australia

T. Rose
e-mail: terry.rose@scu.edu.au

L. Van Zwieten (✉) · D. Herridge · A. Cowie
School of Environmental and Rural Science, University of New England, Armidale, NSW, Australia
e-mail: lukas.van.zwieten@dpi.nsw.gov.au

D. Herridge
e-mail: david.herridge@une.edu.au

A. Cowie
e-mail: annette.cowie@dpi.nsw.gov.au

T. Rose
Southern Cross GeoScience, Southern Cross University, Lismore, NSW, Australia

A. Cowie
NSW Department of Primary Industries, Armidale, NSW, Australia

faba bean yield by addressing P nutrition and ameliorating Al toxicity.

Keywords Boron · Rhodic ferralsol · Field assessment · Lime · Compost · Molybdenum · Natural ^{15}N isotope abundance · Phosphorus

Introduction

Nitrogen (N) is a key driver of plant productivity and N_2 fixed by nodulated legumes is estimated to contribute 33–46 Tg N annually to global agricultural systems (Herridge et al. 2008). Declining levels of organic matter in soils results in a reduced capacity to supply N for crop growth (Dalal and Mayer 1986) and, as a result, crop, fodder and shrub legumes continue to play a critical role in maintaining or improving soil N status (Peoples et al. 1995). In addition to fixing N, legumes contribute to soil carbon (C) via release of root exudates and tissue residues, act as a disease- and pest-breaks in cereal-based rotations, and can enhance the phosphorus (P) nutrition of subsequent crops through undefined mechanisms (Karpenstein-Machan and Stuelpnagel 2000; Nuruzzaman et al. 2005; Rose et al. 2010).

While nodulated legumes offer a promising option as a rotation crop in farming systems, N_2 fixation by rhizobia-legume symbioses is often limited by soil constraints such as acidity, which affects more than 1500 Mha worldwide (Graham and Vance 2000). In addition, the binding of key nutrients including P and molybdenum (Mo) in acid soils (Smith et al. 1997) limits plant uptake of these minerals with further consequences for legume productivity. The deleterious effects of P deficiency in particular to reduce biological N_2 fixation and uptake of soil mineral N by legumes has been well established (e.g., Giller and Cadisch 1995; Graham and Vance 2000).

While liming can overcome the constraints of acid soils and increase legume growth, the accessibility of lime may be a limitation to its use in low-input farming systems. In contrast, biochar (pyrolysed organic matter) has been shown to increase N_2 fixation in acid soils (Rondon et al. 2007; Guerna et al. 2015) and may be a viable option for low-input farming systems by exploiting locally-available feedstocks (Cowie et al. 2012). Rondon et al. (2007) attributed the enhanced N_2 fixation in common bean (*Phaseolus vulgaris* L.) with biochar application in their controlled-environment study to increased boron (B) and Mo availability. The authors also suggested that higher potassium (K), calcium (Ca), and P availability were factors, although higher pH, reduced mineral N and aluminium (Al) saturation may have contributed also, but to a lesser extent. However, given that the acid soil in the study of Rondon et al. (2007) was limed prior to sowing, and that the biochar contained significant quantities of P, Mo and B, it is difficult to attribute the changes in legume growth and N_2 fixation to any long term influence of the biochar on soil properties as opposed to a simple fertilisation effect over the 75-day-study. Indeed, the authors conclude that longer-term field studies were required to better understand the impacts of biochar of N_2 fixation. A more recent glasshouse study by Guerna et al. (2015) demonstrated that biological N_2 fixation increases following biochar amendment, resulting from increased plant P uptake which was associated with a 360 % increase in mycorrhizal colonisation. In contrast, Mia et al. (2014) suggested that improved biological N_2 fixation in red clover (*Trifolium pratense* L.) following biochar amendment resulted from improved K nutrition in a moderately acid soil. The authors suggest this benefit could be short term only due to the relatively high availability of K from the biochars used.

The aim of the present study was therefore to investigate the impact of biochar on legume growth and N_2 fixation in an acidic soil in the field over several seasons and to elucidate any causal mechanisms for enhanced legume performance. In addition to overcoming nutrient deficiencies in acid soils, a number of other possibilities may also explain any enhanced N_2 fixation due to biochar application. Further, the presence of free ammonium (NH_4^+) or nitrate (NO_3^-) in soils can inhibit N_2 fixation (Herridge and Betts 1988). Biochar addition can reduce mineral N concentrations through a range of mechanisms (Cayuela et al. 2014; Clough et al. 2013) and this reduction in free soil NO_3^- and NH_4^+ may enhance the percentage of N_2 fixed by legumes grown with biochar amendments.

As part of a broad program to assess the potential of biochar to enhance legume productivity in the field, we investigated the impact of well-characterised and contrasting biochars, in comparison to a commercially available compost, lime and nil amendment controls, on the growth, grain yield and N_2 fixation of nodulated faba bean (*Vicia faba* L.). The field studies were undertaken on an acidic ferralsol, a soil type that occupies 750

Mha of tropical and sub-tropical agriculture worldwide (FAO 1998). These acidic soils often present constraints in supply of P to crops due to the complexation of phosphate with Al and iron (Fe) oxides, clays and organic matter (Moody 1994). To elucidate causal mechanisms we resolved key changes in soil parameters and plant nutrient uptake as a result of amendment addition to establish correlations between these parameters and legume growth and N_2 fixation. By growing a maize crop in the first season after amendment addition (and between subsequent faba bean crops) to nullify any immediate nutritional benefits (particularly P, Mo and B) from the addition of the amendments, we were able to focus on the longer-term impacts that biochar properties have on soil parameters and legume growth. Resolving the causal factors affecting N_2 fixation and legume growth on the acidic ferralsol following biochar, compost and lime amendments should enable better prediction of the effects of these amendments across a broader range of environments.

Materials and methods

Organic amendments

Two biochars, one from poultry shed waste and the other from papermill residue, were produced by Pacific Pyrolysis Ltd., Somersby NSW Australia, using a continuous 300 kg h^{-1} pilot slow-pyrolysis unit. Poultry shed waste was obtained from a commercial poultry producer and papermill residue was collected from an Australian paper mill previously described in Van Zwieten et al. (2010). Both biomass feedstock's were pyrolysed at a highest treatment temperature (HTT) of 550 °C, at a heating rate of 5–10 °C min^{-1} and with a maximum residence time at HTT of 45 min. The compost was a commercial product, certified to Australian Standards AS 4454 (Composts, soil conditioners and mulches).

Total carbon (TC) concentration of the compost was 21 %, compared with 38 and 42 % for paper mill (PM) and poultry litter (PL) biochars, respectively (Table 1). Total organic carbons (TOCs) were less than TCs for the compost and the two biochars, with the PL biochar showing the largest reduction. Total N concentrations were 2.5 and 2.6 % for the two biochars, compared with 1.2 % for the compost. The mineral N concentrations were low for all three organic amendments, with almost 100 % of the mineral N present as ammonium. Total P, citrate-soluble P and total K were highest in the PL biochar compared to PM biochar and compost.

The PL biochar and PM biochar had pH values of 8.9 and 6.8 respectively, with liming values of 8.8 and 18 % that of agricultural lime ($CaCO_3$) (Table 1). Compost had a liming value of 6.3 %. The PL biochar had 44 cmol NH_4OAc extractable K, and 13 cmol NH_4OAc extractable Na. The PM biochar had lower extractable cations, and was dominated by Ca. Similarly, extractable Ca dominated in the compost. The PL biochar contained relatively high levels of the heavy metals copper (Cu), chromium (Cr), zinc (Zn) and arsenic (As), compared with the PM biochar and compost, while the compost contained relatively high levels of lead (Pb).

Study site and experimental design

The 3 years cereal-legume rotation experiment had amendments applied in December 2007 and was conducted on a Rhodic Ferralsol (described in detail in Macdonald et al. 2014) at the Wollongbar Primary Industries Institute, NSW, Australia (28.49.34″S, 153.23.5″E; elevation 140 m). In summary, the soil is fine-textured and iron-rich with kaolinite, gibbsite and goethite mineralogy. It has a bulk density of 1 g cm^{-3} and a CEC of 22 meq(+) 100 g^{-1}. The phosphorus buffering index of the soil was high at 483 (Slavich et al. 2013). The climate is sub-tropical with a summer-dominant rainfall. Three months before the commencement of the trial, the site was prepared by mowing existing pasture and removing most of the above ground biomass. Glyphosate was then sprayed at the recommended rate and, after 2 weeks, the site was rotary hoed to 100 mm depth of soil. The site was rotary hoed again immediately prior to application of amendments to control weeds. The field site was surveyed and plots 5 × 4 m marked out in a grid with three replicate blocks of ten treatments.

The experiment was designed to investigate the mechanisms from which single application of poultry litter (PL) biochar or papermill (PM) biochar (application rate 10 t ha^{-1} dry wt) impacted growth, grain production and biological N_2 fixation in winter-grown faba bean crops. The three other treatments were compost (25 t ha^{-1} wet wt, equivalent to 13 t ha^{-1} dry wt), agricultural lime (3 t ha^{-1}) and nil amendment. The five soil treatments were combined with the presence or

Table 1 Chemistry of the compost and biochars used in the field study

Chemical measure	Unit	Compost	PM biochar	PL biochar
Total C	%	21	38	42
Total organic C	%	19	35	31
Total N	%	1.2	2.5	2.6
Total P	%	0.29	0.65	1.8
Total K	%	0.66	0.41	2.8
pH ($CaCl_2$)	pH units	7.8	6.8	8.9
$CaCO_3$ equivalent	%	6.3	18	8.8
EC	Ds/m	2.2	1.3	6.1
KCI-extractable NH_4^+	mg kg^{-1}	8.1	3.3	9.4
KCI-extractable NO_3^-	mg kg^{-1}	<0.2	<0.2	0.45
Water -soluble P	mg kg^{-1}	170	490	1400
Citrate -insoluble P	mg kg^{-1}	1000	1600	1200
Citrate-soluble P	mg kg^{-1}	1730	4400	15,000
NH_4OAc extractable cations				
Al	cmol(+) kg^{-1}	<0.03	0.19	<0.03
Ca	cmol(+) kg^{-1}	22	14	4.3
K	cmol(+) kg^{-1}	14	5.7	44
Mg	cmol(+) kg^{-1}	11	3.4	2.8
Na	cmol(+) kg^{-1}	6.8	2	13
Other elements				
Al	%	1.2	0.8	0.25
As	mg kg^{-1}	<3	<3	110
B	mg kg^{-1}	13	10	64
Ca	%	2.6	4	11
Cd	mg kg^{-1}	<0.9	<0.9	<0.9
Co	mg kg^{-1}	17	3.4	2.7
Cr	mg kg^{-1}	64	14	190
Cu	mg kg^{-1}	56	79	310
Fe	%	2.9	0.53	0.37
Mg	%	0.5	0.31	0.69
Mn	mg kg^{-1}	570	190	550
Mo	mg kg^{-1}	4.7	1.9	2.2
Na	%	0.17	0.14	0.61
Ni	mg kg^{-1}	20	5.6	11
Pb	mg kg^{-1}	46	6.4	<1.7
S	%	0.19	0.18	0.42
Se	mg kg^{-1}	<6.6	<6.6	<6.6
Zn	mg kg^{-1}	190	190	550

absence of fertiliser (urea 400 kg ha^{-1}, single superphosphate 300 kg ha^{-1} and muriate of potash 140 kg ha^{-1}, equivalent to 184 kg N ha^{-1}, 26 kg P ha^{-1} and 70 kg K ha^{-1}), applied prior to sowing sweet corn (*Zea mays* L.) across all plots in the summers before, between and following the two faba bean crops. Amendments were applied in December 2007 and the faba bean crop was sown approximately 6 months after application and again in the following season, i.e., 18 months after application. There was no fertiliser applied to the faba bean crops. The site was rotary hoed to incorporate the soil amendments into the 0–100 mm soil layer. The nil amendment plots were also rotary hoed. All fertilisers were subsequently surface applied.

The 10 treatments, i.e., five soil treatments × 2 fertiliser levels, were allocated to plots in a randomised complete block layout with three replicates. Soil bulk densities across the various treatments in the 0–100 mm profile ranged between 1.00 and 1.02 and were not statistically different at 3 months after the application of amendments (data not shown).

Each plot was mechanically sown with six rows of corn in November 2007, 2008 and 2009 and harvested in February 2008, 2009 and 2010. Above ground biomass was removed shortly after harvest (data not presented). Faba bean (cv Cairo) was sown in May in 2008 and 2009 at rates of 100 kg seed ha^{-1}. Prior to sowing, the faba bean seed was inoculated at the recommended rate with commercial Group F inoculant containing *Rhizobium leguminosarum* bv. *viciae* strain WSM1455 (Herridge et al. 2014).

Plant and grain sampling and assessment of N_2 fixation

Faba bean biological N_2 fixation was determined using the natural ^{15}N abundance technique (Unkovich et al. 2008). Plants were sampled at maximum shoot biomass, i.e., late pod-fill (Schwenke et al. 1998). For each plot, a 1 m row of faba bean was harvested to ground level together with an adjacent 1-m row of planted oats (*Avena sativa*) which was used as the non N_2-fixing reference. All plant materials were dried in a dehydrator at 70^0C for 48 h, ground using a Culatti Micro Hammermill fitted with a 1 mm screen, then sub-sampled for analysis. The acid extractable elements in biomass were determined according to USEPA 6010 using a Varian 720-EC ICP-OES, Inductively Coupled Plasma, Optical Emission Spectrometer (ICP-OES). Grain yield was determined by harvesting four rows per plot (equivalent to the width of one pass) using a Wintersteiger small-plot harvester. Measurements were converted to a per-hectare basis.

Subsamples of dried and ground material from the late pod-fill sampling were analysed for %N and $\delta^{15}N$. The subsamples (*ca*. 5 mg) were precisely weighed using a 5-place balance into tin capsules and submitted to the University of California Stable Isotope Facility for analysis by a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The percentage of legume N derived from biological N_2 fixation (%Ndfa) was estimated using standard equations (Unkovich et al. 2008) and a *B* value of −0.39‰ (Schwenke et al. 1998). Total N_2 fixed by faba bean was calculated by multiplying %Ndfa by shoot N (kg ha^{-1}) by a constant of 1.4 to account for below-ground N (Herridge et al. 2008).

Soil sampling and analysis

On six occasions during the study, soil samples were taken to monitor effects of soil amendments and fertiliser on soil properties. Three soil cores (50 mm diameter) were taken from each plot (0–100 mm), air-dried, composited and passed through a 1 mm sieve prior to analysis. Soil and biochar chemical analyses were done in a National Association of Testing Authorities Australia (NATA) facility accredited to ISO17025.

Total C and N were measured by Dumas combustion using an Elementar vario MAX CN analyser with combustion chamber set at 900 °C and oxygen flow rate of 125 ml min^{-1}. The pH was measured in 0.01 M $CaCl_2$ (1:5) according to method 4B2 (Rayment and Higginson 1992). Solubilised cations were assessed using 1 M NH_4OAc described in method 15E1 (Rayment and Higginson 1992) and analysed with a Varian 720-EC ICP-OES, Inductively Coupled Plasma, Optical Emission Spectrometer (ICP-OES). Liming values of amendments were measured as carbonate equivalent using method 19A1 (Rayment and Higginson 1992). The acid extractable elements and metals were determined with the ICP-OES according to USEPA 6010. Plant available Mo in soil was measured using method 12 B 1 (Rayment and Lyon 2010). The method is based on a 0.01 M EDTA extraction buffered to pH 8.6 with NH_4HCO_3. Biochars and compost were tested for total P, water-soluble P, citrate-soluble and -insoluble P according to AOAC Official Methods 977.01 and 963.03 (AOAC International 2000).

Plant analysis

Plant material (0.25 g of dried and ground plant material) was digested in a teflon vessel by microwave using

H_2O_2/HNO_3 according to Wu et al. (1997). Diluted extracts were analysed using a Varian 720-EC ICP-OES, Inductively Coupled Plasma, Optical Emission Spectrometer (ICP-OES).

Statistical analysis

Linear mixed models were used to estimate and compare the various attributes measured for each amendment and season. The model treated each attribute as a response to fixed effects defined by a five-level factor (soil amendment), a two-level factor (fertiliser) and a factor for the interaction between those terms. Where seasonal data were available the fixed effects were extended to include seasons and the interaction between seasons and the other factors. Each model included random effects to estimate variation due to the replicate blocks and, when seasonal data was available, a random effect to include variation due to individual plots.

An analysis of variance was derived from each model in order to conduct null hypothesis significance tests for each factor. The average of each attribute at all combinations of soil amendment, fertiliser and season was predicted together with an estimate of standard error (SE). Formal statistical comparison of the averages was enabled by calculation of least significant difference (LSD) at the 5 % level of probability. Variance heterogeneity due to seasons was observed with some attributes clearly showing different levels of variation within seasons. When that was the case, an extra variance component was included in the model in order to inflate the corresponding standard errors.

To investigate the interaction between the N_2 fixation observations and selected soil and plant yield attributes from the 2008 crop, a principal components analysis was conducted and presented as a biplot. Nine key attributes were identified as soil factors: pH, EDTA available Mo, total C, available Al, and plant factors being grain yield, N fixed by the crop, total P, B and Mo uptake by the crop. The 10 (soil amendments and fertiliser) by 9 (attributes) matrix of averages was created, centered and scaled so that each attribute had mean zero and variance of one over the amendments. Principal component scores and loadings were calculated from this matrix and presented as a biplot with attributes represented by arrows and amendments represented by points. A brief guide to interpreting the biplot is that attributes with arrows in similar direction tend to be positively correlated. Attributes with arrows of opposite direction tend to be negatively correlated. Attributes with arrows at right angles tend to have zero correlation. Similarity of amendments with respect to all attributes is indicated by proximity of their points and the degree of association between amendments and attributes can be indicated by projecting a perpendicular line from the treatment to the attribute vector. All data analyses and statistical graphs were constructed using the statistical package R (R development core team 2012).

Results

Corn yields during three seasons

In 2008 there was no significant effect of amendments on fresh cob yields in unfertilised plots, with yields ranging 17.1–20.2 t ha^{-1} (Table 2). Likewise within fertilised plots, amendments had no impact on yield. However, in comparing across fertilised and unfertilised treatments, fertiliser with lime and biochar amendments yielded significantly ($p \leq 0.05$) higher than the non-fertilised control. All biomass (cob and stover) was removed from plots, resulting in the removal of 193–265 kg N ha^{-1} in unfertilised plots and>300 kg N ha^{-1} in fertilised plots (Supplementary Table 1). The removal of other key nutrients at harvest in 2008 included 19–31 kg P ha^{-1}, 70–136 kg K ha^{-1}, 52–82 kg Ca ha^{-1}, 4–7 kg Mg ha^{-1} and 8–13 kg S ha^{-1} (Supplementary Table 1).

Corn cob yields in 2009 were unaffected by addition of amendments applied in December 2007 in the unfertilised treatments, but lime, PM biochar and PL biochar treatments resulted in significantly higher yields than the control treatment in the presence of fertiliser (Table 2). Further, the PM biochar treatment had significantly higher cob yields than the compost treatment. In 2010 there was no effect of any amendment on cob yields regardless of fertiliser treatment.

Yields of faba bean and nutrient uptake

Significant differences between nil amendment and the four soil amendments, either with or without applied fertiliser, were observed for biomass and grain yields, shoot fixed and total N contents and shoot P content in 2008, while there were no differences between any of the five treatments for shoot B content. Control

Table 2 The effect of amendments and fertiliser treatments on fresh weight of corn cobs (t ha^{-1}) over three seasons. Average standard error of the means was 2.40 (2008), 1.49 (2009) and 2.04 (2010)

	2008		2009		2010	
	Nil fertiliser	Plus fertiliser	Nil fertiliser	Plus fertiliser	Nil fertiliser	Plus fertiliser
Nil amendment	17.1	19.4	22.4	19.4	16.9	16.9
Compost	19.5	20.3	22.5	22.9	16.2	17.9
Lime	20.1	23.0	23.9	24.8	15.5	19.5
PM biochar	20.2	24.4	24.1	27.3	19.2	16.4
PL biochar	21.1	23.5	25.4	23.6	18.8	17.3
LSD (p=0.05)	5.54		4.05		5.49	

treatments showed significantly lower shoot Mo content than all amendments except the PL biochar treatment in the absence of fertiliser in 2008, yet in the plus fertiliser treatment shoot Mo content was significantly higher in the control treatment than all other treatments ($p \le 0.05$; Table 3). In the 2008 season in the absence of fertiliser, compost and PM biochar amendments resulted in significantly higher biomass yields than the PL biochar amendment, and significantly higher grain yields than the lime treatment ($p \le 0.05$). In terms of nutrient uptake in 2008, PL biochar resulted in significantly lower shoot N contents compared to PM biochar and compost treatments in the absence of fertiliser, but no differences in shoot P or B content were observed among amendments. Interestingly, shoot Mo content was significantly higher in the lime treatment compared to other amendments in the presence or absence of fertiliser. In the fertilised plots in 2008, all amended plots had significantly higher shoot P content and biomass and grain yields than control plots, but there was no difference among amendments for any yield or nutrient uptake parameters (Table 3).

The wet weather following the sowing of the second faba bean crop in May 2009 (350 mm in May and 150 mm in June) led to severe infection of all plots with chocolate spot (*Botrytis fabae*). This was then compounded by severe moisture stress when<50 mm rain fell from July to September (Supplementary Fig. 1), leading to extremely poor growth in all plots (grain yields in the nil fertiliser treatment of 0.29 t ha^{-1} in 2009 compare to 1.78 t ha^{-1} in 2008; Table 3). The only significant treatment effects observed in 2009 were significantly higher shoot biomass yields in all amended plots compared to control plots in the fertilised treatment, and significantly higher shoot biomass yields in PM biochar plots compared to control plots in the absence of fertiliser.

Faba bean N_2 fixation

The $\delta^{15}N$ values were similar for the nil and plus fertiliser treatments, but varied substantially between the two seasons particularly for the non N_2-fixing reference, oats (Table 4). The $\delta^{15}N$ values for faba bean ranged −0.12–0.98‰ in 2008 and 1.24–2.92‰ in 2009, compared with $\delta^{15}N$ ranges for the non N_2-fixing oats of 3.22–4.59‰ (2008) and 6.54–12.21‰ in 2009.

The proportion of faba bean shoot N derived from N_2 fixation (%Ndfa) was high for all treatments with average estimates across all treatments of 77 % in 2008 and 72 % in 2009 (Table 4). Overall, the lime and PM biochar amendments resulted in the highest %Ndfa values with the compost amendment the lowest, although the only significant difference ($p<0.05$) was the higher %Ndfa in PM biochar in the fertilised treatment in 2009 (89 %) compared to the compost treatment (62 %). The amount of N_2 fixed in the aboveground biomass (fixed shoot N content) was significantly lower in controls than for all amended plots with or without fertiliser in 2008 (76 kg ha^{-1} for unfertilised vs 84 kg ha^{-1} for fertilised treatment; Table 3). In the absence of fertiliser, fixed shoot N was significantly lower in the PL biochar plots than all other amended plots, while in the presence of fertiliser the compost plots had significantly lower fixed shoot N than all plots with other amendments ($p \le 0.05$; Table 3). Data from 2009 had high variability and no significant differences were detected.

Table 3 Effect of biochar, compost and lime on faba bean yield and shoot nutrient content

Amendment	Shoot DM ($t\ ha^{-1}$)		Grain yield ($t\ ha^{-1}$)		Fixed shoot N content ($kg\ ha^{-1}$)		Total shoot N content ($kg\ ha^{-1}$)		Shoot P content ($kg\ ha^{-1}$)		Shoot B content ($g\ ha^{-1}$)		Shoot Mo content ($g\ ha^{-1}$)	
	Nil fertiliser	Plus fertiliser	Nil fertiliser	Plus fertiliser	Nil fertiliser	Plus fertiliser	Nil fertiliser	Plus fertiliser	Nil fertiliser	Plus fertiliser	Nil fertiliser	Plus fertiliser	Nil fertiliser	Plus fertiliser
2008														
Nil	3.67	4.15	1.78	2.20	76	84	91	111	11.7	14.4	29.1	55.6	1.43	5.91
Lime	7.14	8.07	3.91	4.53	199	199	214	239	21.6	30.2	52.7	64.3	5.05	4.78
Compost	8.34	8.21	4.53	4.38	177	136	241	221	31.1	30.9	55.7	52.6	2.58	3.03
PM biochar	7.89	8.01	4.25	3.94	185	225	232	255	28.4	26.8	57.7	55.6	2.89	3.02
PL biochar	6.89	7.83	3.98	3.88	128	186	172	219	30.2	33.9	54.2	53.3	2.30	3.15
LSD (p=0.05)	1.20		1.12		46		54		9.1		28.1		1.08	
2009														
Nil	1.27	0.80	0.41	0.29	24	16	31	22	na	na	na	na	na	na
Lime	2.10	2.47	0.83	0.74	23	36	43	44	na	na	na	na	na	na
Compost	2.31	2.81	0.70	0.90	34	28	47	55	na	na	na	na	na	na
PM biochar	2.89	2.44	1.06	0.83	49	33	59	58	na	na	na	na	na	na
PL biochar	2.19	3.00	0.66	0.90	34	37	49	56	na	na	na	na	na	na
LSD (p=0.05)	1.54		0.79		46		54							

Note na is not analysed. Shoot refers to total above-ground biomass

Table 4 Effects of soil amendments on $\delta^{15}N$ (‰) of above-ground faba bean and non N_2-fixing reference (oats) and estimated %Ndfa of faba bean, all growing in an acidic ferralsol during 2008 and 2009

Amendment	Faba bean $\delta^{15}N$ (‰)		Oats $\delta^{15}N$ (‰)		Ndfa (%)	
	Nil fertiliser	Plus fertiliser	Nil fertiliser	Plus fertiliser	Nil fertiliser	Plus fertiliser
2008						
Nil	0.45	0.48	3.51	3.83	78	79
Lime	−0.12	0.27	3.22	3.79	93	84
Compost	0.73	0.98	3.60	3.57	73	62
PM biochar	0.47	0.07	3.80	4.59	80	89
PL biochar	0.57	0.25	3.30	3.76	74	85
LSD (p=0.05)	0.84		1.40		23.2	
2009						
Nil	1.45	1.24	9.68	7.57	79	79
Lime	2.92	1.30	10.24	8.80	61	81
Compost	2.05	2.78	8.62	7.12	72	55
PM biochar	1.36	2.36	12.21	8.68	84	66
PL biochar	1.56	2.25	6.54	7.97	70	67
LSD (p=0.05)	1.79		5.06		31.8	

Soil chemistry

Analysis of the soils just prior to sowing of the first, i.e., 2008, faba bean crop *ca*. 7 months following application of soil amendment revealed differences among treatments in EDTA extractable Mo, pH and Bray P (Table 5). Amendment with PM biochar resulted in significantly higher available Mo compared to either control or compost in plots that did not receive fertiliser. In fertilised plots, the limed treatment had significantly lower Mo than either the PM or PL biochar treatments, but these biochar treatments were not different to the control.

In both fertilised and unfertilised control plots, the pH was low (pH=4.3) at the time of sowing the 2008 faba bean crop. All soil amendments significantly increased soil pH in the absence of fertiliser, but only the lime, compost and PM biochar increased pH in the presence of fertiliser. A significant acidification trend was observed across the 4 crop cycle, except in the unamended controls, which were generally significantly lower than amendments (Fig. 1). The PL biochar had the least influence on soil pH of the four amendments.

Poultry litter biochar had significantly higher Bray P in both fertilised and unfertilised plots, compared to controls or other amendments (Table 5). There were no statistical differences between the other amendments. In the absence of fertiliser, there was a decline in available P in the PL biochar plots, but levels still remained significantly higher than for the other treatments during the sampling period (Fig. 2). In the presence of applied fertilisers, there was no significant decline in available P during the sampling period. Poultry litter biochar again had the greatest influence on available P in soil. Fertilised plots tended to have higher Bray P than plots that did not receive fertiliser.

The control and lime amendments had higher nitrate levels in the fertilised plots compared to the unfertilised plots, but there were no differences between soil amendments. There were no treatment effects on soil NH_4^+ concentrations (Table 5).

Bi-plot showing association of yields with various parameters

Biological N_2 fixation was very closely related to plant Mo uptake (Fig. 3). This was influenced by liming and fertiliser. Yield of faba bean, however, was most closely related to plant P and B uptake, as well as soil pH and EDTA-extractable Mo concentration. Amendment with PM biochar had the

Table 5 Soil chemistry before sowing faba bean into an acidic ferralsol in April 2008

Soil amendment	EDTA Mo ($\mu g\ kg^{-1}$)	pH (1:5 $CaCl_2$)	Bray P ($mg\ kg^{-1}$)	NO_3^-N ($mg\ kg^{-1}$)	NH_4^+-N ($mg\ kg^{-1}$)	Exch Al (cmol(+) kg)	Exch K (cmol(+) kg)	Exch Ca (cmol(+) kg)	Total C (%)
Nil fertiliser									
Nil	48	4.3	20.3	16.3	4.10	1.2	0.31	3.6	4.90
Lime	54	5.0	18.7	18.3	3.77	0.18	0.33	7.4	4.77
Compost	48	4.9	26.0	16.0	3.77	0.20	0.65	7.4	5.33
PM biochar	59	5.2	26.0	18.7	4.10	0.13	0.43	8.4	5.27
PL biochar	57	4.8	51.7	21.3	3.83	0.34	0.77	5.6	5.37
Plus fertiliser									
Nil	52	4.3	24.7	39.7	4.00	1.1	0.49	3.9	4.93
Lime	45	5.0	21.3	34.7	3.67	0.17	0.41	7.5	4.97
Compost	50	4.8	32.3	27.0	4.70	0.26	0.63	7.6	5.60
PM biochar	60	5.3	31.7	26.3	4.77	0.08	0.43	8.6	5.33
PL biochar	57	4.4	50.7	27.0	4.23	0.54	0.68	5.3	5.17
LSD (p=0.05)	11	0.45	9.26	16.60	1.62	0.33	0.21	2.4	0.44

strongest influence over these parameters although lime with fertiliser application, PL biochar and compost also had some influence. High exchangeable Al was linked closely to nil amendment controls which had significantly lower pH values compared to amendments (Table 5).

Discussion

A significant and well-defined constraint to productivity of legumes and efficacy of biological N_2 fixation is soil acidity and the related problems of Al- and Mn-toxicity, and deficiency of Mo, Ca, and P (Graham and Vance

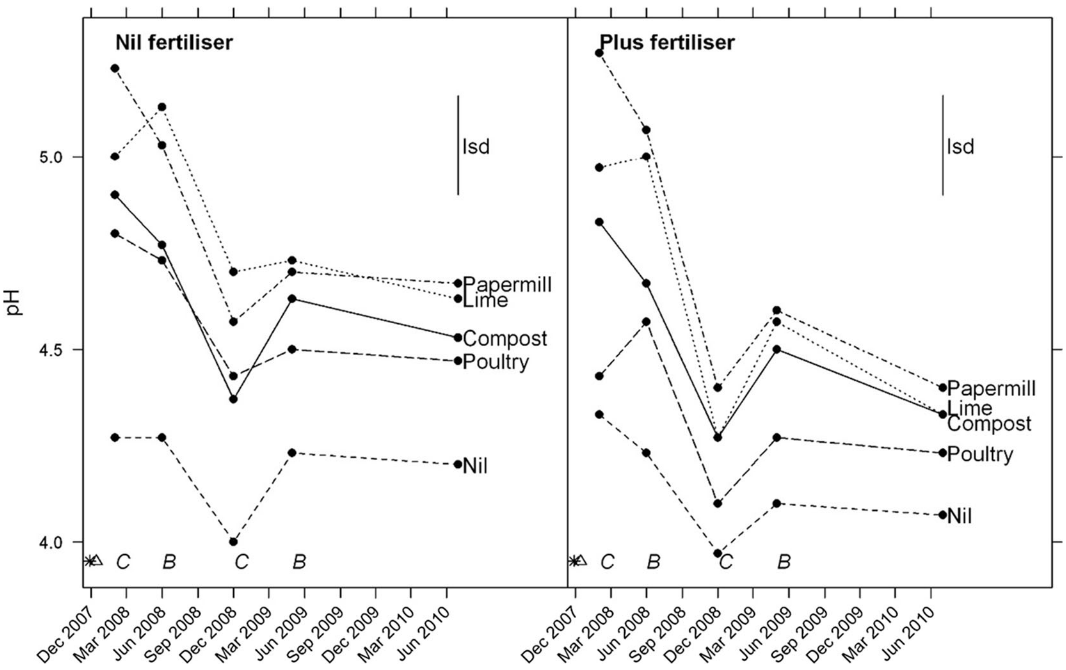

Fig. 1 Average soil pH under each amendment for two crop rotations. * indicates date of amendment, and the triangle indicates sowing of the first corn crop. C (corn) and B (bean) indicate standing crop when samples were taken. Vertical bar span the least significant difference at 5 % critical value for comparing amendments at each observation date

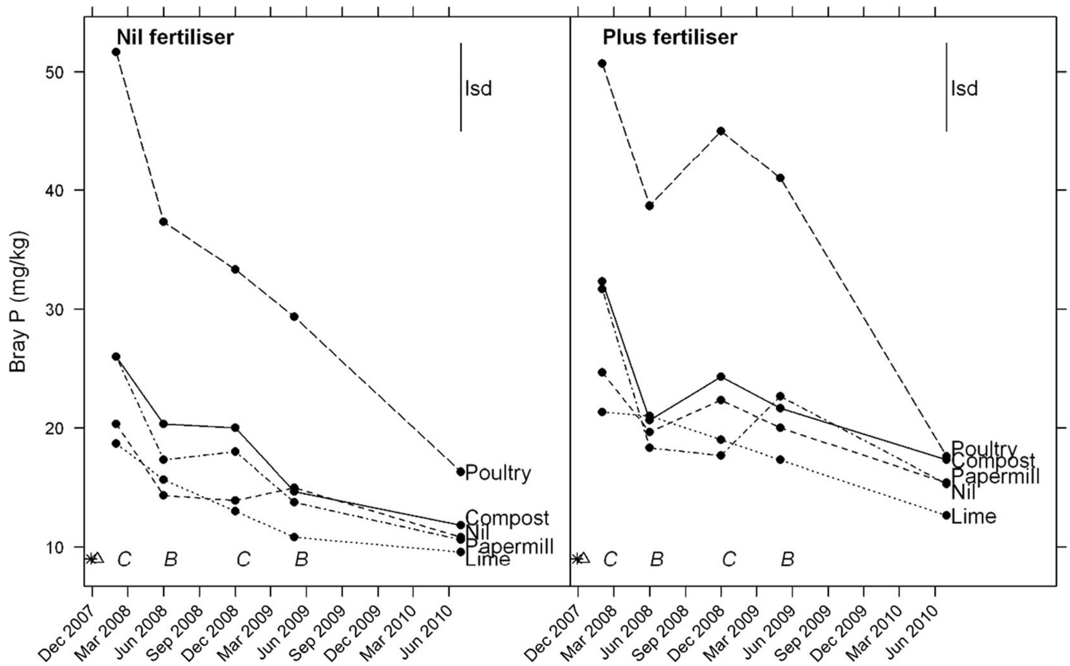

Fig. 2 Average soil Bray P under each amendment for two crop rotations. * indicates date of amendment, and the triangle indicates sowing of the first corn crop. C (corn) and B (bean) indicate standing crop when samples were taken. Vertical bar span the least significant difference at 5 % critical value for comparing amendments at each observation date

2000). Acidity limits both survival and persistence of nodule bacteria in soil, and the process of nodulation itself. The rhodic ferralsol used in this study, while having good physical properties, had a low pH, low available Mo and high exchangeable Al, all of which are well defined constraints to successful legume production. It is possible that some of these constraints (e.g., Ca and P deficiency) may have been overcome in the 2008 faba bean crop as a result of a flush of nutrients following the conversion of a pasture system to a cropping system in early 2008. However, we contend that this is unlikely given that 19–31 kg P ha^{-1} and 52–82 kg Ca ha^{-1}were removed from plots following the first (2008) corn crop (Supplementary Table 1). The fact that faba bean grain yields were higher in the control plots that received NPK fertiliser than control plots without fertiliser (2.20 *vs* 1.78 t ha^{-1}; Table 3) further suggests that nutrient limitations existed. The 2009 faba bean crop suffered from moisture stress and infection by chocolate spot. We attribute this poor growth of faba bean (biomass reduced by over 100 % compared to the previous season) to moisture and disease stress rather than any specific depletion of nutrients or pH effect. Indeed, the pH of the soil in the 2009 faba bean crop in the compost and biochar treatments were not significantly different to the pH of the 2008 faba bean crop, when these treatments resulted in the highest yields in the unfertilised plots (Table 3). This assertion is further supported by the fact that maize yields in the following season (2010) did not show any drastic yield reductions compared to previous maize crops (Table 2). As such, the discussion focusses on the growth and biological N_2 fixation of the 2008 faba bean crop.

Fixation of N_2 in 2008 (shoot fixed N content; Table 3) was most strongly associated with the Mo status of shoot tissue (Fig. 3), supporting the results of earlier studies on acid soils that have found strong correlations between legume Mo uptake and N_2 fixation (Johansen et al. 2007; Rondon et al. 2007). However, rectifying Mo deficiency in the earlier studies was achieved by the addition of Mo fertiliser (Johansen et al. 2007) or a biochar that contained significant Mo that was apparently available to plants over the 75 days growth period (Rondon et al. 2007). In the field trials conducted in the present study, Mo supplied in the amendments could not explain the enhanced Mo nutrition of the legumes and corresponding increases in %Ndfa and therefore shoot fixed N content. The

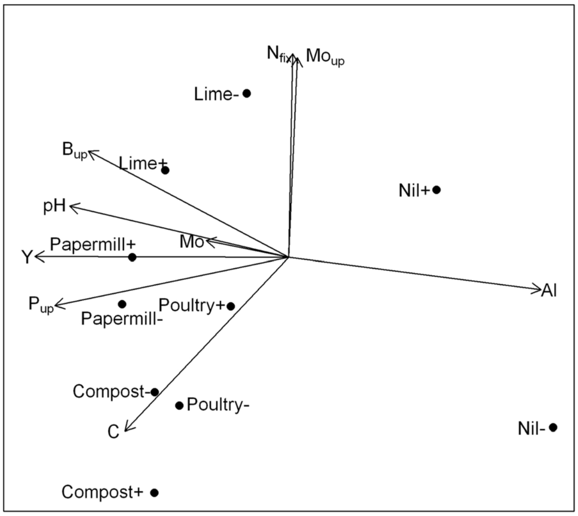

Fig. 3 Bi-plot of the first two principal components calculated from the average 2008 season faba bean yield (Y), N_2 fixed by the crop (N_{fix}), P, B and Mo shoot uptake and associated soil parameters; exchangeable aluminum (Al), soil carbon (C), EDTA molybdenum (Mo) and pH. Amendments are indicated by name in combination with presence (+) or absence (-) of fertiliser. Arrows in similar direction tend to be positively correlated. Attributes with arrows of opposite direction tend to be negatively correlated. Attributes with arrows at right angles tend to have zero correlation. Similarity of amendments with respect to all attributes is indicated by proximity of their points and the degree of association between amendments and attributes can be indicated by projecting a perpendicular line from the treatment to the attribute vector

compost (at 13 t ha^{-1}, dry weight equivalent) had the highest concentration of Mo (Table 1) and supplied substantially more Mo to soils than the lime or biochar amendments. Despite this, the compost treatment resulted in lower %Ndfa (Table 4). Given that the liming treatment resulted in a significantly greater shoot Mo content than all other amendments regardless of fertiliser application (except the nil amendment in the presence of fertiliser, Table 3) and that lime had a strong association with Mo uptake and N_2 fixation (Fig. 3), enhanced soil Mo availability via increased pH appears the most plausible explanation. Molybdenum availability in acidic soils is typically limited due to sorption to Fe- and Al-oxides, with maximum sorption in the range pH 4–5 (Smith et al. 1997). The increase in pH ($CaCl_2$) in the rhodic ferralsol from 4.3 in the unamended soil to 5.0 or greater in the lime and PM biochar treatments (Table 5) presumably enhanced the bioavailability of native Mo in the soil.

Despite this contention, it is acknowledged that soil N status may have influenced the %Ndfa among treatments to some degree because of the different types and amounts of N applied in the various amendments. While is it well established that high soil mineral N can inhibit leguminous biological N_2 fixation (Herridge and Betts 1988), uncertainties exist with the influence of organic N application via plant biomass, compost, or indeed biochar. The cultivation of a maize crop prior to the sowing of the faba bean crop removed much of the readily available N (193–350 kg N/ha exported in removed corn biomass, Supplementary Table 1). This was reflected in the concentrations of NH_4^+ and NO_3^- in the soil prior to sowing the 2008 faba bean crop, where no significant differences were detected amongst amendments (Table 5). However, N mineralisation rates in compost vary widely depending on the source and properties of the compost (Hartz et al. 2000). For example, Pu et al. (2012) recently found negligible N mineralisation from compost added to a sub-tropical ferrosol, while other reports vary from 10 % (Wen et al. 1995) to 50 % (Buchanan and Gliessman 1991) mineralisation over a cropping season. Assuming this range of mineralisation, 16–78 kg N/ha may have been available to the faba bean crop in the 2008 growing season, and this may have contributed to the lower %Ndfa in the compost treatment. In contrast, while the biochars used in our study contained 2.5 and 2.6 % total N for PM biochar and PL biochar respectively, it is unlikely that any major quantity of this N would mineralise in the short term. Using similar biochars to our current study (paper sludge and poultry litter), Singh et al. (2012) reported minimum turnover times for the biochar carbon of 100 years, and as temperature of production increased from 400 to 550 °C, residence time increased significantly. This further suggests that pyrogenic N within biochar may remain relatively unavailable and thus not play an important role in supplying mineral N to the soil and reducing biological N_2 fixation of a legume growing in that soil.

Interestingly, while Mo uptake was the major driver for N_2 fixation, a number of other factors contributed to legume grain yield production in the acid soil. Grain yield was strongly associated with pH, P uptake and to some extent, B uptake (Fig. 3). Macdonald et al. (2014), using a similar soil type, demonstrated that biochars have the ability to increase soil pH resulting in relatively large gains in biomass attributed to improved P nutrition and alleviated Al toxicity. It should be noted here that soil characteristics are important in effecting this outcome, as Macdonald et al. (2014) also showed nil effects on a neutral vertisol as well as negative impacts on plant growth due to increased electrical conductivity in an acidic arenosol. The compost added an estimated 819 kg ha^{-1} lime equivalents, PL biochar 880 kg ha^{-1} lime equivalents and PM biochar 1.8 t ha^{-1} lime equivalents. In-field effects on pH, however, were similar between the limed plots (equivalent to 3 t ha^{-1} agricultural lime) and PM biochar (Fig. 2), suggesting the longer-term liming effect of biochar amendments may be underestimated using the laboratory analysis described in Rayment and Lyon (2010). In previous work (Van Zwieten et al. 2010), PM biochar was shown to have a range of acid neutralising sites based on oxides, hydroxides and carbonate, thus our addition of 3 t ha^{-1} lime into soil was designed to match the anticipated in-field pH response to PM biochar. This was achieved with closely matching soil pH values across the project timeline (Fig. 1). While the PL biochar had the highest P input from all amendments (Table 1), resulting in sustained increases in soil Bray P (Fig. 2), shoot biomass yields were significantly less than the compost and lime treatments in 2008 in the unfertilised plots (Table 3). This may be due to the relatively limited influence PL biochar had on soil pH (Fig. 1). This suggests that both alleviatied Al toxicity and improved P nutrition were required for higher yields. Similarly, Guerna et al. (2015) showed that growth and %Ndfa of common bean (*Phaseolus vulgaris* L.) were closely correlated to plant P uptake in a degraded and slightly acid humic acrisol. The authors surmised that the increased P uptake, however, resulted mostly from improvements in mycorrhizal colonization, with a lesser direct contribution from improved soil pH and nutrient status.

Conclusions

Overcoming the major nutritional constraints to legume production in acid soils over the mid- to longer- term relies on increasing Mo uptake into shoots via amelioration of soil pH. The improvement in crop yields rely on addressing additional constraints such as P availability, especially in high P fixing soil, and overcoming Al toxicity. The study here has demonstrated that biochars can provide an important liming effect in addition to their immediate fertiliser value. Given that the acid-neutralising capacity of biochars depends on both the feedstock and pyrolysis temperature, it may be worth exploiting this to produce biochars with a high acid-neutralising capacity in low-input farming systems constrained by acid soils. The causal mechanisms for enhanced N_2 fixation and crop productivity identified in the present study differed from those identified in short term glasshouse studies. This highlights the need for further field studies over multiple seasons to understand the longer-term impacts of biochar and other amendments on addressing constraints to biological N_2 fixation and legume productivity.

Acknowledgments This work was co-funded as part of an Australian Government Caring for our Country grant in collaboration with Richmond Landcare (Tony Walker) and the NSW Department of Primary Industries. Scott Petty and Barry Outerbridge are acknowledged for managing the field site. Craig Hunt and his team are thanked for their assistance in analysis and technical input.

References

AOAC International (2000) Official methods of analysis, 17th edn. AOAC Int, Gaithersburg, MD

Buchanan M, Gliessman SR (1991) How compost fertilization affects soil nitrogen and crop yield. BioCycle 32:72–77

Cayuela ML, Van Zwieten L, Singh BP, Jeffery S, Roig A, Sánchez- Monedero MA (2014) Biochar's role in mitigating soil nitrous oxide emissions: a review and meta-analysis. Agric Ecosyst Environ 191:5–16

Clough TJ, Condron LM, Kammann C, Muller C (2013) A review of biochar and soil nitrogen dynamics. Agronomy 3:275–293

Cowie AL, Downie A, George BH, Singh BP, Van Zwieten L, O'Connell D (2012) Is sustainability certification of biochar the answer to environmental risks? Pesq Agrop Brasileira 47: 637–648

Dalal RC, Mayer RJ (1986) Long term trends in fertility of soils under continuous cultivation and cereal cropping in southern Queensland. I. Overall changes in soil properties and trends in winter cereal yields. Aust J Soil Res 24:265–279

FAO (1998) Distribution of ferralsols. http://www.fao.org/ag/agl/agll/wrb/wrbmaps/htm/ferralso.htm (Accessed 11 April, 2012)

Giller KE, Cadisch G (1995) Future benefits from biological nitrogen fixation: an ecological approach to agriculture. Plant Soil 174:255–277

Graham PH, Vance CP (2000) Nitrogen fixation in perspective: an overview of research and extension needs. Field Crop Res 65:93–106

Guerna DT, Lehmann J, Thies JE, Enders A, Karanja N, Neufeldt H (2015) Partitioning the contributions of biochar properties to enhanced biological nitrogen fixation in common bean

(*Phaseolus vulgaris*). Biol Fertil Soils. doi:10.1007/s00374-014-0990-z

Hartz TK, Mitchell JP, Giannini C (2000) Nitrogen and carbon mineralization dynamics of manures and composts. HortSci 35:209–212

Herridge DF, Betts JH (1988) Field evaluation of soybean genotypes selected for enhanced capacity to nodulate and fix nitrogen in the presence of nitrate. Plant Soil 110:129–135

Herridge DF, Peoples MB, Boddey RM (2008) Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311:1–18

Herridge DF, Hartley E, Gemell LG (2014) Rhizobial counts in peat inoculants vary amongst legume groups at manufacture and with storage: implications for quality standards. Plant Soil 380:327–336

Johansen C, Musa AM, Rao JVDKK, Harris D, Ali MY, Shahidullah AKM, Lauren JG (2007) Correcting molybdenum deficiency of chickpea in the high barind tract of Bangladesh. J Plant Nutr Soil Sci 170:752–761

Karpenstein-Machan M, Stuelpnagel R (2000) Biomass yield and nitrogen fixation of legumes monocropped and intercropped with rye and rotation effects on subsequent maize crop. Plant Soil 218:215–232

Macdonald LM, Farrell M, Van Zwieten L, Krull ES (2014) Plant growth responses to biochar addition: an Australian soils perspective. Biol Fertil Soils 50:1035–1045

Mia S, van Groenigen JW, van de Voorde TFJ, Orama NJ, Bezemer TM, Mommer L, Jeffery S (2014) Biochar application rate affects biological nitrogen fixation in red clover conditional on potassium availability. Agric Ecosyst Environ 191:83–91

Moody PW (1994) Chemical fertility of krasnozems: a review. Aust J Soil Res 32:1015–1041

Nuruzzaman M, Lambers H, Bolland MDA, Veneklaas EJ (2005) Phosphorus uptake by grain legumes and subsequently grown wheat at different levels of residual phosphorus fertiliser. Aust J Agric Res 56:1041–1047

Peoples MB, Herridge DF, Ladha JK (1995) Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production? Plant Soil 174:3–28

Pu G, Bell M, Barry G, Want P (2012) Estimating mineralisation of organic nitrogen from biosolids and other organic wastes applied to soils in subtropical Australia. Soil Res 50:91–104

R Development Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Rayment GR, Higginson FR (1992) Australian laboratory handbook of soil water chemical methods. Inkata Press, Melbourne, Australia

Rayment GE, Lyon DJ (2010) Soil chemical methods- Australasia. CSIRO Publishing, Collingwood, Australia

Rondon MA, Lehmann J, Ramirez J, Hurtado M (2007) Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. Biol Fertil Soils 43:699–708

Rose TJ, Hardiputra B, Rengel Z (2010) Wheat, canola and grain legume access to soil phosphorus fractions differs in soils with contrasting phosphorus dynamics. Plant Soil 326:159–170

Schwenke GD, Peoples MB, Turner GL, Herridge DF (1998) Does nitrogen fixation of commercial, dryland chickpea and faba bean crops in north-west New South Wales maintain or enhance soil nitrogen? Aust J Exp Agric 38:61–70

Singh BP, Cowie AL, Smernik RJ (2012) Biochar carbon stability in a clayey soil as a function of feedstock and pyrolysis temperature. Environ Sci Technol 46:11770–11778

Slavich PG, Sinclair K, Morris SH, Kimber SWL, Downie A, Van Zwieten L (2013) Contrasting effects of manure and green waste biochars on the properties of an acidic ferralsol and productivity of a subtropical pasture. Plant Soil 366:213–227

Smith KS, Balistrieri LS, Smith SM, Severson RC (1997) Distribution and mobility of molybdenum in the terrestrial environment. In: Gupta UC (ed) Molybdenum in Agriculture. Cambridge University Press, Cambridge, pp 23–46

Unkovich MJ, Herridge D, Peoples M, Cadisch G, Boddey RM, Giller KE, Alves BJR, Chalk P (2008) Measuring plant associated nitrogen fixation in agricultural systems. ACIA R, Canberra, ACT, Australia

Van Zwieten L, Kimber S, Morris S, Chan YK, Downie A, Rust J, Joseph S (2010) Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. Plant Soil 327:235–246

Wen G, Bates TE, Voroney RP (1995) Evaluation of nitrogen availability in irradiated sewage sludge, sludge compost and manure compost. J Environ Qual 24:527–5

Wu S, Feng X, Wittmeier A (1997) Microwave digestion of plant and grain reference materials in nitric acid or a mixture of nitric acid or a mixture of nitric acid and hydrogen peroxide for the determination of multi-elements by inductively coupled plasma mass spectrometry. J Anal At Spectrom 12: 797–806