Biol Fertil Soils
DOI 10.1007/s00374-006-0152-z

ORIGINAL PAPER

Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions

Marco A. Rondon · Johannes Lehmann · Juan Ramírez · Maria Hurtado

Received: 14 December 2005 / Revised: 28 September 2006 / Accepted: 29 September 2006

Abstract This study examines the potential, magnitude, and causes of enhanced biological N_2 fixation (BNF) by common beans (*Phaseolus vulgaris* L.) through bio-char additions (charcoal, biomass-derived black carbon). Bio-char was added at 0, 30, 60, and 90 g kg^{-1} soil, and BNF was determined using the isotope dilution method after adding ^{15}N-enriched ammonium sulfate to a Typic Haplustox cropped to a potentially nodulating bean variety (CIAT BAT 477) in comparison to its non-nodulating isoline (BAT 477NN), both inoculated with effective *Rhizobium* strains. The proportion of fixed N increased from 50% without bio-char additions to 72% with 90 g kg^{-1} bio-char added. While total N derived from the atmosphere (NdfA) significantly increased by 49 and 78% with 30 and 60 g kg^{-1} bio-char added to soil, respectively, NdfA decreased to 30% above the control with 90 g kg^{-1} due to low total biomass production and N uptake. The primary reason for the higher BNF with bio-char additions was the greater B and Mo availability, whereas greater K, Ca, and P availability, as well as higher pH and lower N availability and Al saturation, may have contributed to a lesser extent. Enhanced mycorrhizal infections of roots were not found to contribute to better nutrient uptake and BNF. Bean yield increased by 46% and biomass production by 39% over the control at 90 and 60 g kg^{-1} bio-char, respectively. However, biomass production and total N uptake decreased when bio-char applications were increased to 90 g kg^{-1}. Soil N uptake by N-fixing beans decreased by 14, 17, and 50% when 30, 60, and 90 g kg^{-1} bio-char were added to soil, whereas the C/N ratios increased from 16 to 23.7, 28, and 35, respectively. Results demonstrate the potential of bio-char applications to improve N input into agroecosystems while pointing out the needs for long-term field studies to better understand the effects of bio-char on BNF.

Keywords Biological N fixation · Boron · Charcoal · Molybdenum · Mycorrhiza · ^{15}N

M. A. Rondon · J. Ramírez · M. Hurtado
Centro Internacional de Agricultura Tropical (CIAT),
A.A 6713, Cali, Colombia

J. Lehmann
Department of Crop and Soil Sciences, Cornell University,
Ithaca, NY 14853, USA

M. A. Rondon (✉)
International Development Research Centre,
250 Albert Street, P.O. Box 8500, Ottawa, ON, Canada K1G 3H9
e-mail: mrondon@idrc.ca

Introduction

Growing evidence indicates that many soils around the world have a presence of charcoal particles [biomass-derived black carbon (C), further called bio-char] in different states of interactions with mineral particles in the soil matrix (Goldberg 1985; Schmidt and Noack 2000). In natural environments, bio-char is added to soils as the residual products of incomplete combustion of biomass from forest or savanna fires (Wardle et al. 1998; Bird et al. 1999). There are, however, several sites where bio-char has been added unintentionally or deliberately as a soil amendment, typically as remnants of charcoal production sites (Chidumayo 1994; Mikan and Abrams 1995; Young et al. 1996; Oguntunde et al. 2004), in home gardens and more systematically in so-called Amazonian Dark Earths; these are ancient, very fertile anthrosols generated by the intervention of indigenous communities throughout the Amazon (Lehmann et al. 2003c). Under conditions of very low-fertility soils such as in savannas from South America

and the Amazon Rainforest, additions of modest doses of bio-char to soil were able to increase plant yield and improve several soil quality indicators (Iswaran et al. 1980; Lehmann et al. 2003a).

However, foliar N concentrations of crops decreased in several studies when bio-char was added to soil (Lehmann et al. 2003a; Rondon et al., unpublished data), and N availability was found to be lower on the black C-rich Amazonian Dark Earths than adjacent soils (Lehmann et al. 2003b). This N limitation in black C-rich soils was not found for legumes, and nodulation (Sylvester-Bradley et al. 1980), as well as occurrence of nodulating plants (Gehring 2003), were significantly greater in forests on Amazonian Dark Earths than on adjacent soils. Legumes also performed better on N-limited soils than grasses after bio-char applications (Rondon et al., unpublished data). These results suggested that biological N fixation (BNF) is enhanced by bio-char amendments. Some evidence is provided by results from Nishio and Okano (1991) who found that BNF determined by N difference was 15% higher when bio-char was added to soil at the early stages of alfalfa development and 227% higher when nodule development was greatest. Several studies indicate that bio-char is an excellent support material for *Rhizobium* inoculants (Pandher et al. 1993; Lal and Mishra 1998). However, detailed studies about the relationship between bio-char additions and BNF have not been published.

It is unclear why BNF increases with bio-char additions. Several possible reasons exist: (1) the N availability in soil is lower due to the high C/N ratio of the bio-char and the resulting N immobilization as indicated from Amazonian Dark Earths (Glaser et al. 2002; Lehmann et al. 2003b); (2) the availability of nutrients other than N (i.e., P, K, Ca, Mg, or micronutrients) and the pH are higher (Tryon 1948; Mikan and Abrams 1995; Lehmann et al. 2003a; Oguntunde et al. 2004); and (3) the bio-char enhances mycorrhizal infection, as it is able to serve as a habitat for extraradical hyphae that sporulate in its micropores due to lower competition from saprophytes (Saito and Marumoto 2002). Root infection by arbuscular mycorrhizae significantly increased by adding bio-char to alfalfa in a volcanic ash soil (Nishio and Okano 1991). Similarly, mycorrhizal infection increased when bio-char was added to soil that was inoculated with spores of *Glomus etunicatum* (Matsubara et al. 1995). No studies exist that relate BNF to the effects of bio-char on soil chemical or biological properties.

Therefore, this study investigates the influence of various levels of bio-char additions on BNF of common beans (*Phaseolus vulgaris* L.) on an acid Oxisol and relates BNF to soil nutrient availability and mycorrhizal infection. It is hypothesized that bio-char improves BNF by common beans due to decreased N availability, increased pH as well as nutrient availability, and greater mycorrhizal infection.

Materials and methods

Soils and experimental details

The experiment was conducted at CIAT's (Centro Internacional de Agricultura Tropical) greenhouses in Cali, Colombia. Average daily temperature is 25°C, and relative humidity is maintained at around 60–70%. Soil sampled from the top 0.2 m of a clay–loam oxisol (Typic Haplustox) from the Matazul research site (4°19′N, 72°39′W) at the Colombian Eastern Planes (Llanos) was used. Roots and visible plant residues were removed, and then the soil was air-dried. Before filling the pots, the soil received a basal dose of fertilizer in the equivalent of 300 kg ha^{-1} of lime, 20 kg P ha^{-1}, and 20 kg N ha^{-1}. Given the very low inherent fertility of the soil, this minimum level of fertilization is required to enable proper plant growth of non-adapted plant species such as common beans (Rao et al. 1998). Four replicated pots per treatment were filled with 2 kg of air-dried soil, and bio-char was added to pots in four rates: 0, 30, 60, and 90 g bio-char per kilogram of soil. The pots were arranged in a completely randomized design.

Bio-char production

Bio-char was produced at the bio-char research laboratory at the National University in Bogota, Colombia from logs of *Eucalyptus deglupta* Blume using a large, temperature-controlled kiln. Temperature was maintained at 350°C and the oxygen level regulated at 15%. Charring time was 1 h, and a charring batch consisted of 20 kg of air-dried logs cut into approximately 0.2-m long pieces. At the time of application, a subsample of bio-char was manually ground to <2-mm mesh. A size distribution analysis, as well as some physicochemical analyses, was performed on a subsample (Table 1). The ground bio-char was added and very well mixed with the soil just immediately before filling the pots. Water was then applied to the pots to reach a 60% field capacity, and the pots were allowed to stabilize during 4 weeks before planting, replenishing evaporated water twice a week.

Plant management

Two accessions of common beans (*P. vulgaris* L.) were used: a variety (Line CIAT BAT 477) known for its high nitrogen fixation ability (Kipe-Nolt and Giller 1993) and a non-nodulating isoline (BAT 477 NN). Using a non-nodulating isoline of the same species is the ideal non-fixing plant control plant required for applying the isotope dilution technique and allows for a very precise quantification of nitrogen fixation (Danso et al. 1993; Giller 2001).

Table 1 Chemical and physical characteristics of the added bio-char

	Bio-char
Total C (g kg^{-1})	823.7
Total N (g kg^{-1})	5.73
pH (H_2O)	7.00
Volatile matter (%)	33.2
Moisture content (%)	1.91
Ash content (%)	0.23
Oxygen content (%)	13.7
P-Bray 2 (mg kg^{-1})	49.5
Total P (mg kg^{-1})	580
Total S (mg kg^{-1})	290
Total Mg (g kg^{-1})	1.31
Total B (mg kg^{-1})	9.35
Total Mo (mg kg^{-1})	1.36
CEC ($mmol_c$ kg^{-1})	46.9
Fraction of material <50 μm (%)	54
Iodine Number (g kg^{-1})	265.5

Four seeds of beans previously inoculated with a peat-based inoculum of effective *Rhizobium* (strain CIAT 899) were planted and allowed to grow for 5 days after germination. Then, the two smaller plants in each pot were removed, and the two most vigorous plants were used for the experiment. Plants were allowed to grow for 75 days until complete pod filling of the more precocious plants. Moisture was maintained in the pots at 50–60% of field capacity by periodical (2- to 3-day intervals) weighing of the pots and replenishment of evaporated water.

Isotopic labeling

Five days after germination, when the weak plants were removed, labeled ammonium sulfate (10 at.% ^{15}N) was added to the soil in water solution (0.026 g ammonium sulfate dissolved in 100 ml of water per pot) at an equivalent dose of 5 kg N ha^{-1}. This small dose of N added to the pots was not expected to affect the N fixation process, which could be reduced when high doses of N are applied (Giller 2001). The solution was homogeneously distributed over the soil surface.

Soil sampling and analyses

At the time of planting, a subsample of soil was taken from each pot using a small core auger (15 mm external diameter) and covering the entire soil depth. A similar intermediate soil subsample was taken 40 days after planting. Samples were used for chemical analyses (KCl-extractable NO_3^- and NH_4^+, pH, and redox potential). At harvest time, a final soil sample was collected after thoroughly mixing the soil once the roots were removed. Nitrate and exchangeable NH_4^+ were extracted with 20 ml 1N KCl for 30 min by shaking 2 g field-moist soil on a reciprocating shaker (Eberbach, USA) at 40 cycles per minute. Nitrate and NH_4^+ in the extract were quantified colorimetrically using a segmented flow analyzer (Skalar Autoanalyzer, Skalar, The Netherlands). Soil reaction was determined with a glass pH electrode (Orion PH meter 9156) and redox potential with a redox electrode (Orion, ORP triode 9179) using a dual pH and redox, Termo Orion meter model 250 (Thermo Electron, Philadelphia, USA). Cation exchange capacity was determined by extracting 5 g soil with 50 ml CH_3COONH_4 (pH 7) for 30 min and titrating with NaOH against a color indicator. In addition to the analyses described above, a Morgan extraction (McIntosh 1969) was performed on the final soil sample (10-g sample in 50 ml 0.72 M CH_3COONa: 0.52 M CH_3COOH at pH 4.8; 30-min shaking), and Ca, Mg, K, Zn, Fe, Cu, and Mn were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Ciros (CCD), Spectro, Germany). The *P* values in the extract were below the detection limit of the ICP and were therefore determined by the molybdene ascorbic acid method (Murphy and Riley 1962). A 10-g soil subsample was used to obtain the number of mycorrhiza spores according to the procedures described by Sieverding (1983).

Plant sampling and analyses

Just before harvest, leaves were tested for chlorophyll levels using a hand-held automated chlorophyll meter (SPAD-502, Minolta, Japan). At harvest time, plants were cut 10 mm above the soil surface. Plants were separated into pods, leaves, and stems. Leaf area was measured immediately after harvest with a leaf area meter (LICOR LI-3100, Licor, Lincoln, USA). Senescent leaves which had fallen during the growing period were collected in each pot and added to the leaves collected at harvest time. Roots were carefully removed from the soil, including the fine roots, then washed repeatedly with deionized water until a complete removal of adhered soil was achieved. A small subsample of the roots (1 g) was used to count mycorrhizal infection levels (Sieverding 1983). The remaining fraction was used for measuring biomass and for analysis. Each plant component was put in a paper bag and dried in an electric oven at 40°C for 2 days. Plants were removed from the drying oven, allowed to cool, and individually weighed for dry biomass. Each component was then finely ground, and a composite weighted sample of 2 g was reconstituted using the appropriate proportions of each component. This reconstituted plant sample was used for chemical and isotopic analysis. Plant and charcoal samples were digested at 200°C for 1 h using a 2:1 mixture of concentrated nitric and perchloric acid (0.5-g soil in 5-ml solution; Zasoski and Burau 1977). The solution was evaporated to dryness,

dissolved in 0.1 N nitric acid and analyzed by ICP-AES as described above. Nitrogen isotope determinations were done by isotope ratio mass spectrometry (Europa Hydra 20/20, PDZ Europa, Northwich Cheshire, UK).

Statistical analyses

Main effects were computed by analysis of variance using a completely randomized design (SAS Institute, Cary, NC). In case of significant effects, individual means were compared by least significant difference test at $P<0.05$ if not indicated otherwise.

Results

Biomass production of the N-fixing beans was significantly higher than that of the non-N-fixing isoline across all levels of bio-char additions (Fig. 1). Bio-char additions significantly increased total biomass production by 39% up to 60 g kg^{-1} bio-char, but decreased biomass to the level of the control with 90 g kg^{-1}. Most of the increase in biomass production by the N-fixing beans was caused by greater leaf biomass (Fig. 1). Whereas total biomass did not change relative to the control at the highest bio-char application rate, biomass of pods continued to increase in N-fixing plants (Fig. 1).

The proportion of N derived from biological N fixation (NdfA, Fig. 2) significantly increased from 50% without bio-char additions to 72% with 90 g kg^{-1} bio-char added to soil. The total N from BNF, however, peaked already at an application of 60 g kg^{-1} due to the low biomass production (Fig. 1; significant main effect $P=0.0059$) and foliar N concentrations (Table 2) at 90 g kg^{-1}. Similarly, leaf area and shoot/root ratios were greatest at 30 and 60 g kg^{-1}, respectively, and not at the maximum bio-char application of 90 g kg^{-1} (Fig. 3).

The N concentrations in plant tissue were significantly lower in the non-N-fixing than in the N-fixing bean variety (Table 2). Additionally, N concentrations of N-fixing beans significantly decreased with greater bio-char applications (Table 2). Plant P, K, Ca, Mg, and B concentrations significantly increased with bio-char applications, whereas tissue S, Zn, Cu, and Mn concentrations did not change, and plant Fe and Al concentrations significantly decreased (Table 2). Molybdenum levels were only detectable in the highest bio-char dose in the N-fixing plants. Concentrations of Mo in plant tissue of the non-nodulating beans, however, were detectable and significantly increased with bio-char additions. In general, tissue concentrations of nutrients other than N were greater in non-N-fixing than in N-fixing beans. This led to a similar total nutrient uptake by N-fixing and non-N-fixing beans (Fig. 3). Similar to the response in biomass production and

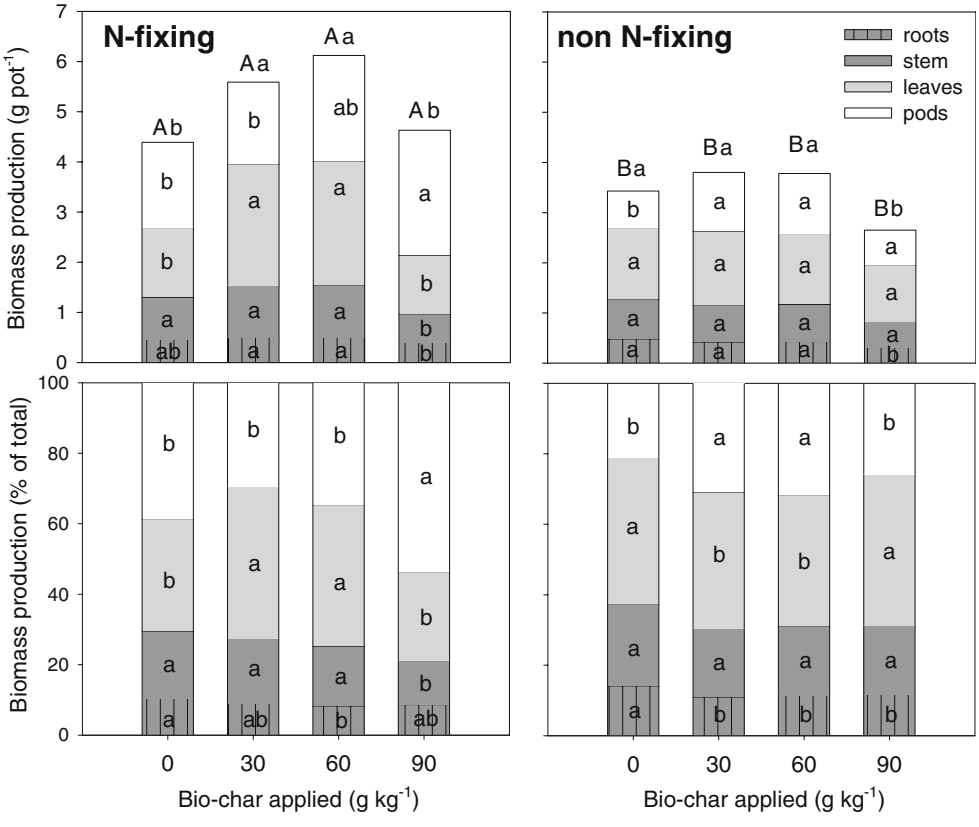

Fig. 1 Biomass production and yield of common beans [*P. vulgaris* L.; N-fixing (BAT477) and non-N-fixing (BAT477NN) strain] grown on a Typic Hapludox as a function of added bio-char; *bars* with the same *small letter* within one plant part and with the same *capital letter* between isolines within the same bio-char application rate are not significantly different at $P<0.05$ ($n=4$)

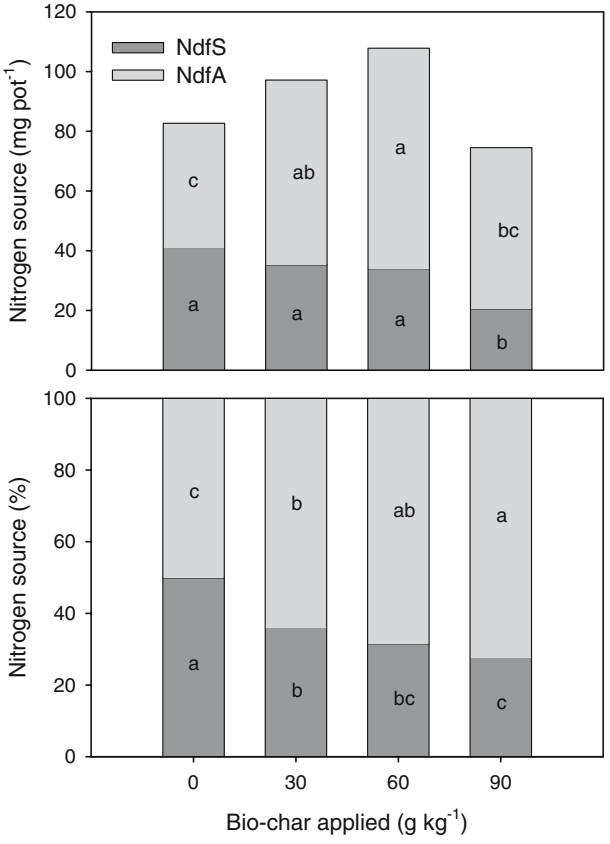

Fig. 2 Proportion of N derived from biological N fixation (NdfA) or from soil (NdfS) by common beans (*P. vulgaris* L.) grown on a Typic Hapludox as a function of added bio-char; *bars* with the same *small or capital letter* are not significantly different at $P<0.05$ ($n=4$)

leaf area, total uptake of most nutrients (except for S, Zn, B, and Mo) was highest at intermediate application rates of bio-char.

The pH significantly increased with greater bio-char additions and was more acid in soils cropped to non-N-fixing beans than to their N-fixing isoline (Table 3). Consequently, Al levels significantly decreased with bio-char additions as well. As a result of the high bio-char applications, the C/N ratio significantly increased by 125% from 15.6 to 35 in the soils with N-fixing plants. Available K and Mg concentrations in soil increased on average by 140 and 70% per 30 g kg^{-1} bio-char addition (equivalent to 48.6 mg C ha^{-1}), respectively. Extractable P concentrations, however, did not increase significantly, and available Ca even decreased slightly (Table 3). The cation exchange capacity only showed a non-significant tendency to increase by 1 to 26% with bio-char additions.

Extractable mineral N was not significantly affected by the bio-char additions ($P=0.51$), but was significantly lower in soil under N-fixing than non-N-fixing plants (Fig. 4). Mineral N significantly decreased on average by 51% throughout the experiment (average over all application and species).

Bio-char additions did not show a discernable effect on the number of spores or on root colonization by mycorrhiza (Fig. 5). With the exception of root colonization of the control, both the number of spores and colonization were significantly greater in N-fixing than in non-N-fixing beans. The species identified were *Enthrophospora* sp., *Glomus* sp., and *Scutellospora* sp.

Table 2 Tissue nutrient concentrations of N-fixing (BAT477) and non N-fixing (BAT477NN) bean isolines (*P. vulgaris* L.) grown with different doses of bio-char

Bean variety	Bio-char (g kg^{-1})	N (g kg^{-1})	P	S	K	Ca	Mg	Fe	Al	Zn (mg kg^{-1})	Cu	Mn	B	Mo
N-fixing	0	18.7a	1.08d	2.09bc	13.0c	8.6cde	1.26d	1.31a	4.10ab	134	43.5	49.0b	1.35e	0.10[b]
	30	17.4ab	1.20d	1.60c	12.9c	7.3e	1.38d	1.23ab	4.19ab	151	46.3	35.6c	1.32e	nd
	60	17.7ab	1.11d	1.60c	14.1bc	8.2de	1.39d	0.79c	2.49d	94	26.6	33.6c	2.16de	0.09[b]
	90	16.0b	1.29cd	1.96bc	17.0a	10.2bc	1.45cd	0.91bc	3.28abc	183	36.0	35.0c	4.01bcd	0.43[b]
Non N-fixing	0	11.6c	1.52bc	2.26b	15.7ab	9.2cd	1.45d	1.34a	4.40a	201	38.9	50.2b	3.34cde	1.63[c]
	30	12.3c	1.78b	2.53b	16.8ab	11.1ab	1.83b	1.10abc	3.74abc	143	39.2	54.3b	6.35ab	2.31[b]
	60	10.5c	1.53bc	2.34b	16.0ab	11.2ab	1.74bc	1.03abc	3.24abc	261	36.5	54.7b	5.69bc	2.07[b]
	90	12.9c	2.22a	3.52a	17.5a	12.3a	2.23a	1.02abc	2.63cd	136	43.1	68.3a	8.25a	3.23[a]
Variety effect[a]		***	***	***	***	***	***	ns	ns	ns	ns	***	***	nd
Bio-char effect[a]		ns	***	**	**	**	***	*	*	ns	ns	ns	**	***

Values within one column followed by the same letter are not significantly different at P<0.05 (N=4).
[a] Main effect of all treatments; *, **, ***, and ns significant at P<0.05, 0.01, 0.001, and not significant, respectively
[b] Only 1–2 replicates were above the detection limit of 0.05 mg kg^{-1}.
ND not detected, *NS* not significant

Discussion

Bio-char effects on BNF

Biological N fixation significantly improved crop performance under our experimental conditions, as biomass production and grain yield were significantly lower in non-N-fixing beans than in N-fixing beans. Nitrogen deficiency was visually apparent in non-N-fixing plants by distinct yellowing already 20 days after planting confirmed by chlorophyll measurements (data not shown). Bio-char additions significantly increased BNF of common beans at all application rates. Evidence for improved BNF by bio-char was also provided for alfalfa (*Medicago sativa* L.) using the N difference and acetylene reduction assay methods (Nishio and Okano 1991). The isotope dilution method employed in our study provided estimates of increases from 50 to 72% of the N in beans to be derived from BNF. This is a significant improvement compared to estimates of 19–27% for common beans on a sandy soil in Mexico (Vásquez-Arroyo et al. 1998), or of 56–67% in Romania (Popescu 1998). The isolines BAT477 have been selected for their high N-fixation ability among common beans (Kipe-Nolt and Giller 1993). It should be noted that the improvements in BNF as well as biomass production (5–39%) are above and beyond the normal productivity achieved by the recommended fertilization practice. Therefore, the effects shown in this greenhouse experiment are a significant improvement of productivity that justify in-depth field studies.

The reason for the improved BNF are most likely a combination of factors related to nutrient availability in soil (Lehmann et al. 2003a,b) and stimulation of plant–microbe interactions (Nishio and Okano 1991; Saito and Marumoto 2002). It is likely that reduced N availability stimulated BNF. Foliar N concentrations decreased with all application rates of bio-char, although the proportion of BNF increased, and total N uptake from soil significantly decreased (Fig. 2). These observations provide strong evidence for a stimulating effect by charcoal to BNF through a reduction of soil N availability. However, the exchangeable NH_4^+ (and NO_3^-) values were not conclusive in spite of some indications that N availability was greater in soils that did not receive bio-char. Because only mineral N was determined, N mineralization from labile organic N pools such as particulate organic N may have limited N availability. Further studies on N dynamics in soils with bio-char additions are required to understand the interactions of organic and mineral N with highly aromatic organic carbon compounds bearing high C/N ratios.

Apart from the stimulating effect of low N availability, increased availability of other nutrients contributed to greater BNF of beans. Foliar concentrations and uptake of B and Mo (Ca and P only slightly) indicated better availability of these elements which are known to increase BNF (Carpena et al. 2000; Giller 2001) when bio-char was added to soil. Specifically, Mo fertilization proved to be the most effective way of increasing BNF and yields in other studies such as of soybeans (Campo and Lantmann 1998) and common beans (Brodrick et al. 1992; de Oliveira et al. 1998), as it is a constituent of the Mo–Fe protein of nitrogenase (Smith 1977). Our data indicate that in the non-nodulating bean isolines, the Mo concentration in plant tissue significantly increased with bio-char additions. In the tissue of N-fixing plants, however, the levels of Mo were, in general, close to detection limits and were detectable only at the highest bio-char dose (90 g kg^{-1}). Molybdenum has been reported as being translocated from leaves and shoots to roots (Gupta 1991), and nodules have been found to have higher Mo concentrations than other plant parts (Jongruaysup et al. 1997). In our experiment, nodules were not separately collected at harvest time, and a large proportion of the nodules may have remained in the soil when extracting the roots, as they tend to easily separate from roots once the plant is mature. If nodules store most of the Mo in plant tissue, then we may have not accounted for such Mo, and we were consequently underestimating Mo levels in the nodulating plant, whereas the non-nodulating plant was not affected by the harvest procedure. The fact that Mo levels were noticeably higher in non-fixing plants at the highest bio-char dose than at other levels also provides an indication that the Mo availability to plants indeed increased with bio-char additions. Lower soil acidity and increased pH by bio-char additions may have also contributed to the greater BNF, as similar effects are commonly observed by liming (Giller 2001). However, BNF significantly increased already at an application rate of 30 g kg^{-1}, whereas the pH did not increase at that level of bio-char additions irrespective of N_2 fixation (Table 3), which suggested that a pH increase contributed to a lesser extent to a BNF improvement in our experiment. Mycorrhizal associations and enhanced nutrient access by beans did not appear to be the cause of greater BNF in our study, as neither root colonization nor number of spores increased with bio-char additions (Fig. 5). This is in contrast to several studies that showed greater mycorrhizal infection of alfalfa (Nishio and Okano 1991) and onion (Matsubara et al. 1995) with bio-char additions, which was explained by bio-char serving as a habitat for extraradical hyphae (Saito

Fig. 3 Leaf area, shoot–root ratio, and nutrient uptake by common beans [*P. vulgaris* L.; N-fixing (BAT477) and non-N-fixing (BAT477NN) strain] grown on a Typic Hapludox as a function of added bio-char; *BC* and *F* indicate significance of the effects of bio-char and N fixation capability, respectively (means and standard errors; $n=4$); Mo uptake of non-N-fixing beans are means of only 1–2 replicates due to some values falling below detection limits (see text)

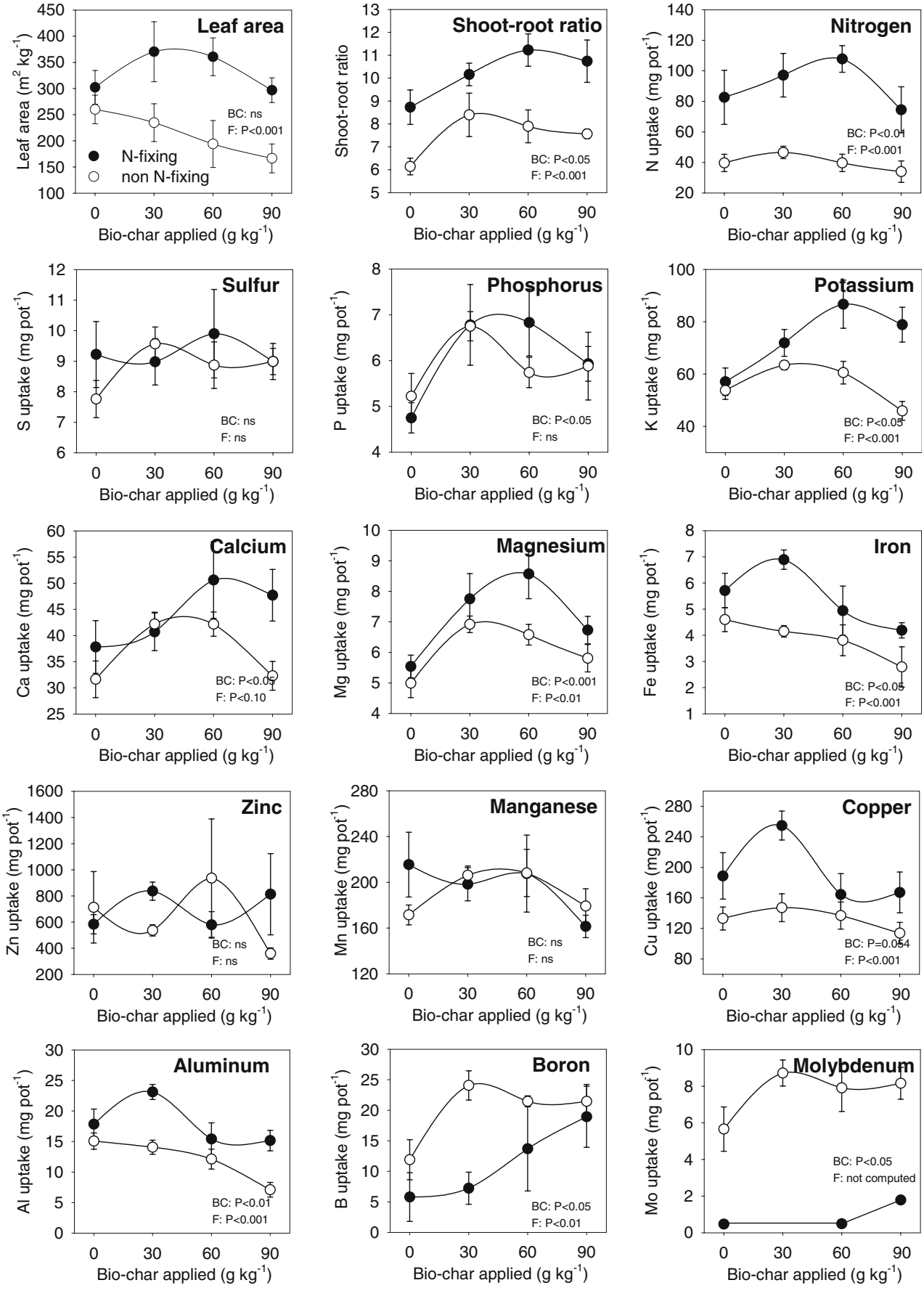
Leaf area
Leaf area (m2 kg-1)
BC: ns
F: P<0.001
N-fixing
non N-fixing
Shoot-root ratio
Shoot-root ratio
BC: P<0.05
F: P<0.001
Nitrogen
N uptake (mg pot-1)
BC: P<0.01
F: P<0.001
Sulfur
S uptake (mg pot-1)
BC: ns
F: ns
Phosphorus
P uptake (mg pot-1)
BC: P<0.05
F: ns
Potassium
K uptake (mg pot-1)
BC: P<0.05
F: P<0.001
Calcium
Ca uptake (mg pot-1)
BC: P<0.05
F: P<0.10
Magnesium
Mg uptake (mg pot-1)
BC: P<0.001
F: P<0.01
Iron
Fe uptake (mg pot-1)
BC: P<0.05
F: P<0.001
Zinc
Zn uptake (mg pot-1)
BC: ns
F: ns
Manganese
Mn uptake (mg pot-1)
BC: ns
F: ns
Copper
Cu uptake (mg pot-1)
BC: P=0.054
F: P<0.001
Aluminum
Al uptake (mg pot-1)
BC: P<0.01
F: P<0.001
Boron
B uptake (mg pot-1)
BC: P<0.05
F: P<0.01
Molybdenum
Mo uptake (mg pot-1)
BC: P<0.05
F: not computed
Bio-char applied (g kg-1)

Table 3 Chemical characteristics of the soil cropped to N-fixing (BAT477) and non N-fixing (BAT477NN) bean isolines (*P. vulgaris* L.) with different doses of bio-char

Bean variety	Bio-char ($g\ kg^{-1}$)	pH (H_2O)	Al ($mg\ kg^{-1}$)	RP (mV)	Total C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	C/N ratio	P ($mg\ kg^{-1}$)	K ($mg\ kg^{-1}$)	Ca ($mg\ kg^{-1}$)	Mg ($mg\ kg^{-1}$)	CEC ($mmol_c\ kg^{-1}$)
N-fixing	0	5.04e	173.3	641c	12.33a	0.791a	15.58	5.17a	94d	1012	28de	108.2
	30	5.08de	140.2	643c	21.21b	0.896b	23.67	4.62ab	219c	370	44c	118.5
	60	5.24c	120.8	663b	27.03c	0.967c	27.97	4.34ab	321b	453	54bc	131.7
	90	5.41b	97.2	664b	38.01d	1.0885c	35.05	4.42ab	451a	667	86a	131.5
Non	0	5.13cde	139.5	665b	11.39a	0.740a	15.38	4.47ab	106d	714	25e	102.5
N-fixing	30	5.17cd	114.5	686a	18.42b	0.867b	21.25	4.39ab	216c	508	43cd	103.4
	60	5.34bc	110.8	680a	22.54c	0.893bc	25.26	2.01c	311b	697	62b	117.0
	90	5.62a	82.3	685a	40.16d	0.951c	42.46	3.58b	489a	653	83a	129.0
Variety effect[a]		***	**	***	ns	ns		***	ns	ns	ns	ns
Charcoal effect[a]		***	***	**	***	**		***	***	ns	***	ns

Values within one column followed by the same letter are not significantly different at $P<0.05$ ($N=4$).
RP redox potential, *Nmin* mineral N (nitrate+ammonium), *CEC* cation exchange capacity
[a] Main effect of all treatments; *, **, ***, and ns significant at P<0.05, 0.01, 0.001, and not significant, respectively.

and Marumoto 2002). Other root–microbe interactions could have contributed to the improvement of BNF by common beans in our study, such as greater microbial release of P due to greater microbial biomass and activity which was frequently found in soils that are rich in charcoal (Zackrisson et al. 1996). However, considering the large increase in B and Mo concentrations and uptake by beans (for Mo only proven for non-N-fixing beans due to concentrations below detection limits), as well as their importance for the enzymes involved in BNF, it appears that improved B and Mo availability contributed the most to an improved BNF in addition to possibly lower N availability. Improvement of the availability of other nutrients, of soil reaction, or of mycorrhizal infection played a less important role.

Bio-char effects on bean productivity

Apart from the stimulating effect of bio-char applications for BNF, bio-char also significantly improved biomass production and yield of common beans (Fig. 1). Such responses confirm earlier results with moong bean [*Vigna radiata* (L.) R. Wilczek], soybean [*Glycine max* (L.) Merr.], and pea (*Pisum sativum* L.) (Iswaran et al. 1980), or with cowpea (*Vigna unguiculata* L.) and rice (*Oryza sativa* L.) (Nehls 2002; Lehmann et al. 2003a). In our study, the improved crop performance was largely an effect of elevated P, K, Mg, Ca, Mo, and B availability as well as higher pH. With the exception of Mo and B, the uptake of all of the nutrients mentioned above declined with the largest application rate, which indicates a very good availability of Mo and B as a result of bio-char applications. In contrast to our data, Lehmann et al. (2003a) did not find an increase in Mg availability but did find an improved Mn and Zn availability. Such differences may be explained by the lower application in the present experiment (30–90 g kg^{-1} compared to 100–200 g kg^{-1} in Lehmann et al. 2003a) for the micronutrients and the well-known differences in base cation concentrations between bio-chars of different origin (Tryon 1948). It is noteworthy that the available K concentrations in soil significantly increased as a response to 30 g kg^{-1} bio-char additions (Table 3), whereas K concentrations in plant tissue did not increase (Table 2). This well-documented positive effect of elevated K avail-

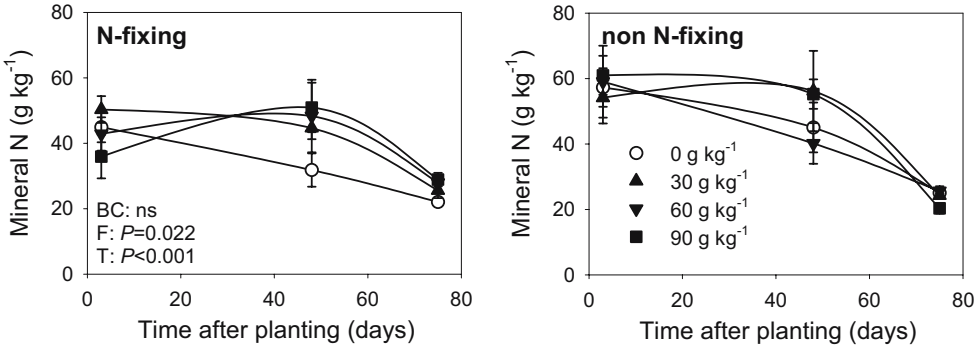

Fig. 4 Dynamics of mineral N $\left(N-NH_4^+ + N-NO_3^-\right)$ in soil cropped to common beans [*P. vulgaris* L.; N-fixing (BAT477) and non-N-fixing (BAT477NN) strain] as a function of added bio-char; *BC*, *F*, and *T* indicate significance of the effects of bio-char, N fixing capability, and time, respectively (means and standard errors; $n=4$)

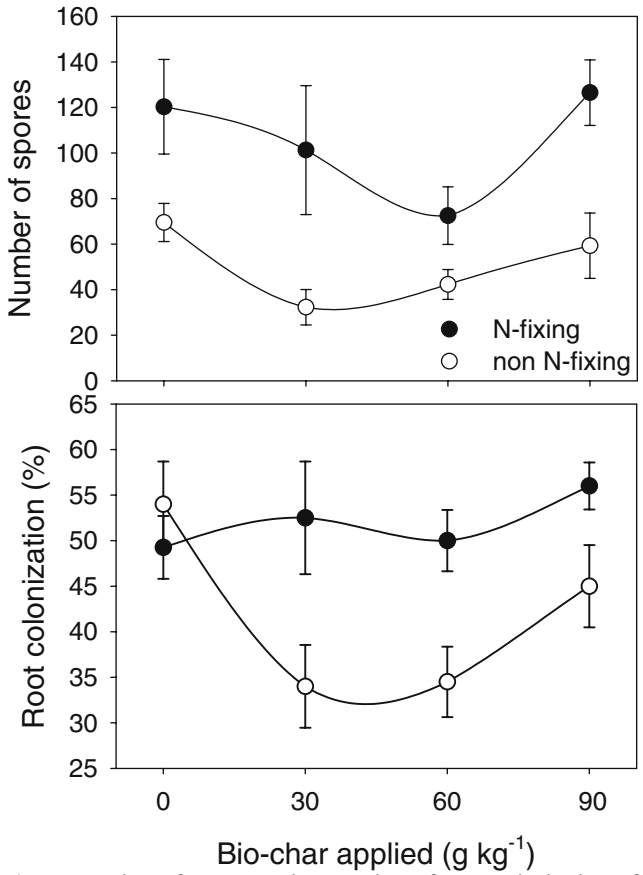

Fig. 5 Number of spores and proportion of root colonization of mycorrhiza of common beans [*P. vulgaris* L.; N-fixing (BAT477) and non-N-fixing (BAT477NN) strain] grown on a Typic Hapludox as a function of added bio-char (means and standard errors; n=4)

ability through bio-char additions (Lehmann et al. 2003a) was able to improve crop growth but did not compensate for the very low K availability of the savanna Oxisol. Similar to Lehmann et al. (2003a), extractable soil P did not significantly increase by bio-char applications, whereas in our study, P uptake and even plant P concentrations increased (Table 2). Phosphorus availability may have been increased in soil, which is not reflected by the soil extraction method.

Threshold of bio-char applications

Whereas the yield of beans increased with higher bio-char applications, biomass production significantly declined above 60 g kg^{-1}. The threshold above which productivity of common beans decreased most likely lay around 60 g kg^{-1}, as leaf area did not increase from 30 to 60 g kg^{-1}, whereas biomass and shoot/root ratios increased, and the uptake of many nutrients peaked at or below 60 g kg^{-1}. This application of 60 g kg^{-1} is equivalent to an application of 121.5 mg bio-char per hectare ha^{-1} or 97.2 mg C ha^{-1} if calculated for the plough layer of 0.15 m with an average bulk density of 1.35 mg m^{-3}.

Similar to biomass production, the increase in BNF did not relate linearly to the increase in bio-char additions. Already at the lowest tested level of bio-char additions (30 g kg^{-1}, equivalent to 60.8 mg bio-char per hectare ha^{-1}), BNF increased by 49% over the unamended control. Adding an additional 30 g kg^{-1} increased BNF only by another 29%, and BNF dropped to 30% above the control when 90 g kg^{-1} were applied.

Whereas BNF showed a tendency to decrease with greater bio-char applications, BNF did not significantly decrease below the values found with the lowest bio-char application. Therefore, a lower BNF alone was not responsible for the decrease in productivity. Nitrogen uptake from soil significantly decreased at high bio-char application rates, and this could be a reason for the lower productivity, as BNF was not able to compensate for the lower soil N uptake (Fig. 2). The foliar N levels were significantly lower than the levels considered to be sufficient for beans (30–40 g kg^{-1}; Bergmann 1986), and N deficiency is likely to have occurred. Low fertility required significant N fertilization even for legumes such as common bean on a Colombian plinthic Kandiudox (Singh et al. 2003). The significantly lower bean yield and tissue N concentrations in non-N-fixing than in N-fixing beans at any bio-char application rate support this interpretation. The C/N ratios (24-35) of the soils amended with bio-char are above the threshold commonly assumed for N immobilization (20; Stevenson and Cole 1999). Other possible reasons for the decrease in productivity with high bio-char applications include allelopathic effects associated with hydrocarbons or toxic levels of heavy metals (not for Ni which was tested; Pb possible) originating from the bio-char. However, conclusive information is not available at present.

Conclusions

Nitrogen fixation significantly improved by moderate rates of bio-char additions. The reason for the improved BNF was mostly an effect of the improved availability of B and likely of Mo, and to a lesser extent, a decreased N availability at C/N ratios of 24-35 and increased availability of K, Ca, and P, and higher pH as well as lower Al saturation. The reason for a drop in BNF as well as biomass production (though not yield) at high bio-char application rates is less clear but may be related to a low N availability and consequently low photosynthate production. These results demonstrate the potential for increasing the N input by BNF into agroecosystems in highly weathered and acid soils by bio-char applications. Future studies should include field experimentation to optimize BNF and explore the sustainability of BNF improvement by bio-char.

References

Bergmann W (1986) Ernährungsstörungen bei Kulturpflanzen. VEB Gustav Fischer Verlag, Jena

Bird MI, Moyo C, Vecnendaal EM, Lloyd J, Frost P (1999) Stability of elemental carbon in a savanna soil. Glob Biogeochem Cycles 13:923–932

Brodrick SJ, Sakala MK, Giller KE (1992) Molybdenum reserves of seed, and growth and N_2 fixation by *Phaseolus vulgaris* L. Biol Fertil Soils 13:39–44

Campo RJ, Lantmann AF (1998) Effects of micronutrients on biological nitrogen fixation and soyabean productivity. Pesqui Agropecu Bras 33:1245–1253

Carpena R, Esteban E, Sarro M, Peñaloza J, Garate A, Lucena J, Zornoza P (2000) Boron and calcium distribution in nitrogen fixing pea plants. Plant Sci 151:163–170

Chidumayo EN (1994) Effects of wood carbonization on soil and initial development of seedlings in miombo woodland, Zambia. For Ecol Manage 70:353–357

Danso SKA, Hardarson G, Zapata F (1993) Misconceptions and practical problems in use of ^{15}N soil enrichment techniques for estimation N_2 fixation. Plant Soil 152:25–52

de Oliveira WS, Meinhardt LW, Sessitsch A, Tsai SM (1998) Analysis of *Phaseolus–Rhizobium* interactions in a subsistence farming system. Plant Soil 204:107–115

Gehring C (2003) The role of biological nitrogen fixation in secondary and primary forests of Central Amazonia. Unpublished PhD thesis, University of Bonn, Germany

Giller KE (2001) Nitrogen fixation in tropical cropping systems, 2nd edn. CAB International, Wallingford

Glaser B, Lehmann J, Zech W (2002) Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—a review. Biol Fertil Soils 35:219–230

Goldberg E (1985) Black carbon in the environment. Wiley, New York

Gupta U (1991) Boron, molybdenum and selenium status in different plant parts in forage legumes and vegetable crops. J Plant Nutr 14:613–621

Iswaran V, Jauhri K, Sen A (1980) Effect of charcoal, coal and peat on the yield of moong soybean and pea. Soil Biol Biochem 12:191–192

Jongruaysup S, Dell B, Bell R, O'Hara G, Bradley S (1997) Effect of molybdenum and inorganic nitrogen on molybdenum redistribution in black gram (*Vigna mungo* L. Hepper) with particular reference to seed fill. Ann Bot 79:67–74

Kipe-Nolt J, Giller K (1993) A field evaluation using ^{15}N isotope dilution method of lines of *phaseolus vulgaris* L. bred for increased nitrogen fixation. Plant Soil 134:178

Lal JK, Mishra B (1998) Flyash as a carrier for *Rhizobium* inoculant. J Res (Birsa Agric Univ) 10:191–192

Lehmann J, da Silva JP Jr, Steiner C, Nehls T, Zech W, Glaser B (2003a) Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. Plant Soil 249:343–357

Lehmann J, Kern DC, German LA, McCann J, Martins GC, Moreira A (2003b) Soil fertility and production potential. In: Lehmann J, Kern DC, Glaser B, Woods WI (eds) Amazonian Dark Earths: origin, properties, management. Kluwer, The Netherlands, pp 105–124

Lehmann J, Kern DC, Glaser B, Woods WI (2003c) Amazonian Dark Earths: origin, properties, management. Kluwer, The Netherlands

Matsubara YI, Harada T, Yakuwa T (1995) Effect of inoculation density of VAM fungal spores and addition of carbonized material to bed soil on growth of Welsh onion seedlings. J Jpn Soc Hortic Sci 64:549–554

McIntosh JL (1969) Bray and Morgan soil test extractants modified for testing acid soils from different parent materials. Agron J 61:259–265

Mikan CJ, Abrams MD (1995) Altered forest composition and soil properties of historic charcoal hearths in Southeastern Pennsylvania. Can J For Res 25:687–696

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36

Nehls T (2002) Fertility improvement of a Terra Firme Oxisol in Central Amazonia by charcoal applications. MSc thesis, University of Bayreuth, Germany

Nishio M, Okano S (1991) Stimulation of the growth of alfalfa and infection of mycorrhizal fungi by the application of charcoal. Bull Natl Grassl Res Inst 45:61–71

Oguntunde P, Fosu M, Ajayi A, Giesen N (2004) Effect of charcoal production on maize yield, chemical properties and texture of soil. Biol Fertil Soil 39:295–299

Pandher MS, Gupta RP, Bhandal BK, Gupta SK, (1993) Studies on growth and survival of *Rhizobium* isolates in different carriers. Indian J Ecol 20:141–146

Popescu A (1998) Contributions and limitations to symbiotic nitrogen fixation in common bean (*Phaseolus vulgaris* L.) in Romania. Plant Soil 204:117–125

Rao I, Miles J, Granobles J (1998) Differences in tolerance to infertile acid soil stress among germplasm accessions and genetic recombinants of the tropical forage grass genus, *Brachiaria*. Field Crops Res 59:43–52

Saito M, Marumoto T (2002) Inoculation with arbuscular mycorrhizal fungi: the status quo in Japan and the future prospects. Plant Soil 244:273–279

Schmidt MWI, Noack AG (2000) Black carbon in soils and sediments: analysis, distribution, implications, and current challenges. Glob Biogeochem Cycles 14:777–793

Sieverding E (1983) Manual of methods for laboratory studies of vesicular arbuscular mycorrhiza. CIAT Technical Document. Cali, Colombia

Singh SP, Teran H, Munoz CG, Osorno JM, Takegami JC, Thung MD (2003) Low soil fertility tolerance in landraces and improved common bean genotypes. Crop Sci 43:110–119

Smith BE (1977) The structure and function of nitrogenase: a review of the evidence for the role of molybdenum. J Less-Common Met 54:465–475

Stevenson FJ, Cole MA (1999) Cycles of soil: carbon, nitrogen, phosphorus, sulfur, micronutrients, 2nd ed. Wiley, New York, USA

Sylvester-Bradley R, De Oliveira LA, De Podestá Filho JA, St. John TV (1980) Nodulation of legumes, nitrogenase activity of roots and occurrence of nitrogen-fixing *Azospirillum* spp. in representative soils of Central Amazonia. Agro-Ecosyst 6:249–266

Tryon EH (1948) Effect of charcoal on certain physical, chemical, and biological properties of forest soils. Ecol Monogr 18:81–115

Vásquez-Arroyo J, Sessitsch A, Martínez E, Peña-Cabriales JJ (1998) Nitrogen fixation and nodule occupancy by the native strains of *Rhizobium* on different cultivars of common bean (*Phaseolus vulgaris* L.). Plant Soil 204:147–154

Wardle DA, Zackrisson O, Nilsson MC (1998) The charcoal effect in boreal forest: mechanisms and ecological consequences. Oecologia 115:419–426

Young MJ, Johnson JE, Abrams MD (1996) Vegetative and edaphic characteristics on relic charcoal hearths in the Appalachian Mountains. Vegetatio 125:43–50

Zackrisson O, Nilsson MC, Wardle DA (1996) Key ecological function of charcoal from wildfire in the boreal forest. Oikos 77:10–19

Zasoski RJ, Burau RG (1977) A rapid nitric-perchloric acid digestion method for multielement tissue analysis. Commun Soil Sci Plant Anal 3:425–436