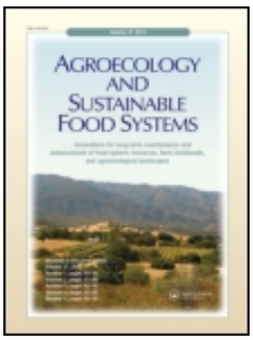

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Variable effects of biochar and P solubilizing microbes on crop productivity in different soil conditions

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ABSTRACT

An expanding body of literature informs that biochar improves soil quality and agricultural productivity. However, there are some reports of little, or even negative, effect of biochar on crop yield, depending on the type of biochar feedstock, pyrolysis process, soil nutrient status, and crop species. Biochar is known to adsorb ammonia and phosphates in soil and facilitate growth and activities of phosphorus (P) solubilizing microbes (PSM), which mobilize P for uptake by plant roots. Using slow-pyrolyzed wood biochar and PSM in different soil conditions in three countries, our experiements show that soil nutrient status is more determinant of beneficial agronomic effect of biochar than the feedstock species and the type of crop. Treatments with biochar and PSM entail significant yield increase in P-deficient soil, whereas in soils with high P content, biochar has no significant effect on crop yield, regardless of addition of PSM. Based on published empirical data as well as our own findings, we also present a mathematical model of plant uptake of bioavailable P at different soil P concentrations, which explains that biochar is ineffective to enhance PSM activity for P mobilization in phosphate-rich soil, but significantly improves crop productivity in P-deficient soil.

KEYWORDS

Biochar; agricultural productivity; soil fertility; soil microbes; phosphorus

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Introduction

Biochar, the product of pyrolysis of biomass, can be produced from any ligno-cellulosic biomass, including brushwood and waste from timber harvest (Wang et al. 2013; Yargicoglu et al. 2015), crop byproducts such as rice and wheat straw (Wang et al. 2013), weedy shrubs and grasses (Mandal et al. 2015), as well as animal manure (Guo et al. 2014), and, therefore, constitutes a relatively inexpensive "input" in agricultural production. Biochar application in agricultural soil is an ancient practice in many parts of the world and is a major component of the fertile Terra Preta ("dark earth") of the Amazon Basin, which sequestered large quantities of carbon in the soil for millennia and sustained productivity of ancient agroforestry of the Amazonian natives

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(Glaser et al. 2001; Lehmann and Joseph 2009). Over the past decade, biochar has attracted major research attention and engendered a large body of evidence of biochar's potential to improve soil quality, thereby, increase agricultural productivity, reduce pH of acidic soils, retain soil moisture, reduce greenhouse gas emission, and store carbon in the soil (Lehmann et al. 2006; Verheijen et al. 2009; Laird 2010; Sohi et al. 2010; Jha et al. 2010; Jeffery et al. 2011; Lehmann et al. 2011; Spokas Cantrell, et al. 2012; Clough et al. 2013; Karer et al. 2013; Fox et al. 2014; Mau and Utami 2014; Novak et al. 2014; Prommer et al. 2014; Wilson 2014; Zhang et al. 2014). However, many of these functions may yield positive, or even negative, agronomic benefits (Spokas, Cantrell, et al. 2012; Biederman and Harpole 2013) depending on the crop species under consideration, soil fertility status, and biochar properties (such as pore size and distribution, ion exchange capacity, nutrient contents), which, in turn, depend on the type of biochar feedstock (wood, manure, or sewage sludge), pyrolysis temperature, rate of heating (fast or slow), and kiln residence time (Novak and Busscher 2012; Spokas, Cantrell, et al. 2012; Mukherjee et al. 2014; Nartey and Zhao 2014; Novotny et al. 2015).

Most of the chemical analyses of low-temperature (<500°C), slow-pyrolysis wood biochar show that total N and bioavailable P contents of ligneous wood biochar do not exceed 0.9 g/kg and 0.7 g/kg, respectively (Table 1). Thus, wood biochar per se adds little nutrients for ready uptake by plants, yet serves to enhance nutrient supply by its ability to retain nutrients in soil and reduce leaching losses through sorption of nitrates and phosphates. Biochar can adsorb up to 20–43% of (5 mg per gram of biochar) ammonium and 19–65% of the phosphate (0.2 mg g^{-1}) in flushed dairy manure in 24 h (Ghezzehei, Sarkhot, and Berhe 2014). More importantly, biochar elicits microbial mobilization of organically and inorganically bound P, which plants can readily utilize (Chan and Xu 2009; Zheng, Sharma, and Rajagopalan 2010; Fox et al. 2014). Although some portion of the sorbed ammonia is available to plants for N uptake (Spokas, Novak, and Venterea 2012; Taghizadeh-Toosi et al. 2012), higher crop yields with biochar amendment are often accompanied by increased availability of P, but not N (Karer et al. 2013).

It is generally understood that porous biochar particles provide an important habitat for soil microbes that facilitate transportation of N and P to the root system of crop plants (Glaser et al. 2002; Lehman, Gaunt, and Rondon 2006; Lehmann and Joseph 2009; Lehmann et al. 2011; Fox et al. 2014). Scanning electron microscopic (SEM) examinations (Lehmann and Joseph 2009; Lehmann et al. 2011; Hammer et al. 2014; Mukherjee et al. 2014; Prommer et al. 2014) have conclusively established that wood biochar pores mostly range between 1 and 15 μm size, sufficient for the arbuscular mycorrhizal (AM) fungi to intrude their

Table 1. Major phytonutrient contents of biochar prepared from slow pyrolysis of wood at low temperatures (300–500°C).

Biochar feedstock	Total N (g/kg)	Total K (g/kg)	Total P (g/kg)	Available P (g/kg)	Reference
Wood	7.6	0.464	0.029		Major et al. (2010)
Eucalyptus	5.73	NA	0.6	0.049	Rondon et al. (2007)
Hardwood residue	1.8	0.017		0.71	McElligoot (2011)
Wood pellets	1.1–1.3	0.8–1.0	0.2–0.22		Laird et al. (2011)
Prosopis sp.	1.12	29.0	1.06		Shenbagavalli and Mahimairaja (2012)
Pepperwood	3	1.0	0.3		Yao et al. (2012)
bamboo	4	3.0	2.4		
Salix sp.	9.1	1.04	1.56	0.04	Prendergast-Miller, Duval, and Sohi (2013)
Salix rosthornii	12.6	NA		0.42–0.65	Zeng et al. (2013)
Eucalyptus	8	0.03	0.02		de Melo Carvalho et al. (2013)
Ponderosa pine	3.53	1.96	0.36	0.004	Chintala et al. (2013)
Eucalyptus	8	0.002	0.002		de Melo Carvalho et al (2013)
Wood	8.1	3.6	0.1		Widowati, Asnah, and Utomo (2014)
Sitka spruce	4.2	NA		0.0036	Troy et al. (2014)
Mixed pine splinter	1.2	3.5	3.5		Melas (2014)
Hardwood pellets	3.0	3.8	0.6		Zhang (2014)
Beech wood chips	4	NA	2.153		Frišták and Soja (2015)
Lantana camara	0.112	0.06	0.003		Mandal et al. (2015)
Woodchips (95% poplar, 5% willow)	< 10	8.7	3.5		Gronwald et al. (2015)
Lops of *Shorea robusta*	0.03	1.4	0.03		
Syzygium cumini	0.12	0.7	0.05		This study
Eupatorium odoratissimum	2.40	2.3	0.1		

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hyphae, which transport P adsorbed to the biochar surfaces. In the soil, bacteria and AM fungi that mobilize soil nutrients for plant uptake, adhere to both exterior and interior (pore) surfaces of biochar particles (Fox et al. 2014; Hammer et al. 2014; Melas 2014; Mukherjee et al. 2014; Prommer et al. 2014). Short-term incubation studies also indicate beneficial effects of biochar on soil microbial community (Mitchell et al. 2015). Biochar, thus, functions as a soil conditioner, enhancing plant growth by sorbing and mobilizing important nutrients, especially P, for plant growth.

Biochar effect on crop productivity is crucially linked to phosphorus solubilizing microbial (PSM) community, which enhances nutrient uptake by plant roots. Enhanced P uptake is particularly important for plants in nutrient-poor soils, such as in the Tropics. As an adaptation to nutrient-poor soil, plant roots host PSM, especially mycorrhizal fungi, which grow on and in plant roots, thereby dramatically increasing the surface area of roots available for exploration and uptake of N and P (Vance 2001). Soil amendment with biochar significantly enhances PSM biomass (Lehmann et al. 2011;

Karer et al. 2013; Fox et al. 2014; Mau and Utami 2014; Zhang et al. 2014), which contributes to the extent of 90% of plant P uptake (van der Heijden et al. 2006; Hammer et al. 2014).

Despite a considerable amount of research that links biochar's sorption chemistry and soil microbial action to agricultural production dynamics (Lehmann et al. 2011; Yao et al. 2012; Crane-Droesch et al., 2013; de Melo Carvalho et al. 2013; Filiberto and Gaunt 2013; Mau and Utami 2014; Soinne et al. 2014; Vaccari et al. 2015), our current understanding of biochar effects in the presence or absence of PSM on crop yield is still limited by the paucity of experimental studies on different crops under similar biochar application regimes in different soil types. We present here results of our multilocation trials of wood biochar combined with PSM, on different types of crops. This report contributes to an understanding of the synergistic role of biochar and PSM in crop yield enhancement and constitutes the first of a series of our experiments with biochar on a range of crops, tested in three countries.

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Materials and methods

Study locations and crop types

We conducted parallel trials on Basudha farm (www.cintdis.org/basudha), located in the district of Bankura, West Bengal, India (23°12′25.6″ N, 87°16′ 54.8″ E); on the experimental station farm of Sita Logistics Ltd. (www.sitalogistics.com) at Thung Yao in the Province of Mae Hong Son, Thailand (19°19′06.79″ N, 98°25′28.75″ E); and in a glasshouse in Chobham, Surrey, UK (51°20′42″ N, 0°37′00″ W).

The farm in India: The farm's topsoil is sandy clay (44% sand, 52% clay, 4% silt) on oxisol substrate. The topography of the area and the farm soil characteristics until June 2009 are described elsewhere (Deb et al. 2012). The farm received no synthetic agrochemicals over the past 20 years. Soil samples were collected at the onset of the experiment in the winter of 2010, and tested in-house, using standard methods cited in the Government of India (2011). The farm soil had a mean pH of 6.1, and electrical conductivity (EC) = 0.15 ms cm^{-1}. The organic matter content (Walkley–Black) of the soil was 3.2%. Soil nutrient levels were low: available nitrogen of 234 kg ha^{-1}, exchangeable potassium (NH_4O-acetate) of 90.7 kg ha^{-1}, and available phosphorus (Olsen) of 2.8 mg kg^{-1}.

The farm in Thailand: The topsoil is primarily silt loam (5% sand, 6% clay, 89% silt) on ultisol substrate. The farm received no chemical inputs over the past 10 years. The farm soil, tested in the winter of 2010, prior to the onset of our experiment, had a mean pH of 6.2 and EC = 0.55 ms cm^{-1}. All soil parameters were tested by Central Laboratory (Thailand) Co. Ltd. (Report

Nos. TR(CM) 54/02544 and TR(CM) 56/15619). The available nitrogen was high (660 kg ha^{-1}), exchangeable potassium (NH_4O-acetate) was medium (154 kg ha^{-1}), and available phosphorus (Olsen) was low, at 10.45 mg kg^{-1}.

Glasshouse in UK: The soil of the experimental tubs was fine textured silt loam (30% sand, 29% clay, 41% silt) on inceptisol substrate. The soil was composed of rich organic compost, procured from an organic farm, and mixed with rock phosphate. The soil parameters were tested in the autumn 2010 by NRM Laboratories of Horsell, Surrey, UK (Report No. 14973/10, Code M330) in August 2010, prior to the onset of the experiment. The soil pH was 6.6, and E.C. = 0.308 ms cm^{-1}. The available N was moderate (NH_4-N = 443.3 kg ha^{-1}), while the levels of available K and P (Olsen) were very high (519.2 kg ha^{-1} and 106.33 mg kg^{-1}, respectively).

The objective of our experiments was to assess the effect of biochar application on the productivity of leaf crops (represented by edible jute), fruit crops (represented by tomato and capsicum), root crops (radish) and grain crops (rice) in different locations (Table 2). The capsicum ("bell pepper") used in England was Roberta F1; the tomato variety was Aastha, and the rice was Shati, a short-duration (ca. 70 days) upland local landrace, procured from Basudha's rice seed bank. The edible jute (in India) and the radish variety (in Thailand) were procured from local farmers.

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Design of study

At the onset of the experiment in June 2010, all plots were treated with 400 g m^{-2} composted cattle manure (equivalent nitrogen content was 12 g m^{-2}), 80 g m^{-2} of green manure, and rock phosphate (RP). In the jute test, RP was applied at 45 g m^{-2} whereas for all other tests, RP was applied at 50 g m^{-2} (20% P_2O_5).

As the objective of agricultural application of biochar, in combination with soil microbes, is essentially to improve crop productivity (Lehman and Joseph 2006; Lehman, Gaunt, and Rondon 2006, 2009; Filiberto and Gaunt 2013; Schulz, Dunst, and Glaser 2013), we designed experiments to compare the net yield effects of biochar addition, with or without the addition of PSM

Table 2. Test crops, field locations, and the year of study.

Type of crop	Leaf crop	Fruit crop		Root crop		Grain crop
Species tested	*Corchorus capsularis*	*Lycopersicon esculentum*	*Capsicum anuum*	*Raphanus sativus*		*Oryza sativa* ssp. *indica*
Variety	Mitha pât	Aastha	Roberta F1	'Chinese'		Shâti
Field location	India	India	England	India	Thailand	India
Year of study	2010–2011	2011–2012	2011–2012	2012–2013	2012–2013	2011–2012

inocula. To test the possibility of interchangeability of effects of PSM with that of biochar addition, we also examined plots inoculated with PSM, with no biochar application. For all crops, separate beds were prepared for three types of treatments (degrees of freedom = 2), namely, (A) biochar + PSM; (B) biochar alone; and (C) PSM alone. The last category (C) serves as the control, because all tropical and temperate agricultural farm soil ecosystems naturally harbor a wide range of AM fungi and/or P solubilizing bacteria.

For the edible jute (*Corchorus capsularis*), 10 earthen pots represented ten replications, in each of which were planted two jute seedlings. For radish in Thailand and capsicum in the UK, plants were grown in 1-m × 1-m and 1.5-m × 1-m plots, respectively. We deployed six replications for each treatment of radish in Thailand and nine replications for capsicum in the United Kingdom. For all other crops, each replication was planted in 2-m × 2-m plots. The overall design of the experiment is described in Table 3.

The three discrete treatments A, B, and C, described above, comprise what we refer to as the Treatment 1 in subsequent analyses. We incorporated a second level of treatment for radish (in Thailand). Each of the Treatments (A, B, and C) was reclassified into two groups: manure fortified with soya residue and without soya residue. Thus, for radish, Treatment 1 (comprised by A, B, and C) was crossed with a second level of treatment (Treatment 2 in subsequent analyses), comprised by addition and no addition of soya (Table 4).

Biochar preparation and application

We used lignin-rich wood as feedstock (Table 5), upon understanding that a) more lignin content of feedstock yields more biochar mass (Novotny et al. 2015), and that b) greater concentrations of lignin in the feedstock tend to increase C recovery (Lehman, Gaunt, and Rondon 2006; Cagnon

Table 3. Replications of three treatments for four crop types.

Treatments	Jute (India)	Tomato (India)	Capsicum (UK)	Radish (India)	Radish (Thailand)	Rice (India)
A. Biochar + PSM	10	5	9	5	6	5
B. Biochar, no PSM	10	5	9	5	6	5
C. PSM, no Biochar	10	5	9	5	6	5

Table 4. Design of experiment with radish in Thailand.

Treatment 1	A	A	B	B	C	C
Biochar	Yes	Yes	Yes	Yes	No	No
PSM	Yes	Yes	No	No	Yes	Yes
Treatment 2	Soya	No soya	Soya	No soya	Soya	No soya

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et al. 2009). In India, we prepared biochar in situ from locally pyrolyzed brushwood, composed of stems and twigs of *Eupatorium odoratissimum* (an exotic invasive shrub) and *Syzigium cumini* of diameter not exceeding 2 cm, harvested from the surrounding scrubland and farm margins. We employed the traditional method of slow pyrolysis of the feedstock in earthen kiln for 12 h. The biochar for use in Thailand was obtained from slow pyrolysis of lops of branches of *Shorea robusta*, collected from the forest and pyrolyzed in a traditional earthen kiln. The biochar used in the UK experiment was obtained from commercially available trimmings (size 200–300 cm^2, 4–10 cm thick) of oak (*Quercus robur*). The biochar was produced by "slow" pyrolysis, low-moderate temperature, and long residence time, which typically yields greater mass of solid biochar than that from "fast" pyrolysis at high (>500°C) temperatures (IEA Bioenergy 2010; Nartey and Zhao 2014). In all three fields, the feedstock consisted of ligneous wood (no leaves), the pyrolysis temperatures were low (350–500° C), and the dose of application was 10 t ha^{-1}. The nature of feedstock and the pyrolysis conditions in the three study fields are summarized in Table 5.

In the experimental plots of treatments A and B, at all three experimental sites, biochar material was pulverized into small (8–12 mm across) particles, which were evenly spread as a layer 10–15 mm thick on the soil surface, and then mixed with the soil to the depth of 6 cm.

PSM inoculation

Phosphorus solubilizing microbes (PSM) in our study comprised by *Pseudomonas* sp., *Bacillus megaterium, B. subtilis*, and arbuscular mycorrizal (AM) fungi *Glomus intraradices*, and *G. mosseae*. The bacteria were obtained from the microbial amendment *Azophos* (Nitrofix Laboratory, Kolkata) containing *Azotobacter chrococcum* and *Bacillus polymyxa*. The colony-forming unit of the bacterial mixture was 3.4×10^9 cells g^{-1} of carrier material. The inoculum was mixed with rice starch at 250 gL^{-1} following the standard

Table 5. Production of biochar from slow pyrolysis used in study.

Country	Feedstock species	Lignin content[a] (%)	Diameter or thickness range (cm)	Temp. (°C)	Residence time (h)
India	Brushwood of (1) *Eupatorium odoratissimum*	48.4	1.0–2.0	350–400	12
	(2) *Syzygium cumini*	40.2			
Thailand	Lops of *Shorea robusta*	40.7	1.5–3.0	350–400	10
UK	*Quercus robur* wood trimmings	25.7[b]	4.0–10.0	400–500	8

[a] See Methods section
[b] *Source*: Obst, Sachs, and Kuster (1988)

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practice in India (Jackson and Ilamurugu 2013; Chatterjee, Jena, and Paul 2013), and dissolved to a concentration of ca. 0.01 g m^{-2}, before spreading on the soil surface of experimental plots of Treatments A and C.

In addition to the bacteria, we applied mycorrhizal inoculation, following the standard practice as described in Miyasaka et al. (2003). Native AM fungi (*Glomus intraradices*, and *G. mosseae*) were grown in a nursery of rice and grasses, maintained for over 4 months from early March to early June 2010. The nursery soil bed was prepared primarily with sand and lateritic soil, and periodically watered. Ten days before the onset of our experiment (June 20, 2010), water was withheld, aboveground biomass of all grasses and rice plants was cut and removed. Roots with basal soil mass were transferred to sterilized trays, air-dried in shade for 2 days, cut into small (<2 cm) pieces. This served as the mycorrhizal inoculum, which was subsequently mixed thoroughly with the treated soil (30% v/v) in each plot of Treatments A and C.

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Identification of microbes

The AM fungi collected from colonized roots and the bacteria in *Azophos* mixture were identified at Nitrofix Laboratories, Banshdroni, Kolkata, and Biotechnology Laboratory for Conservation, Kalikapur, Kolkata.

Determination of lignin content

Wood samples (0.175 g) were digested in 72% H_2SO_4 at 47°C for 1 h, under vigorous stirring. After complete digestion, the sample was autoclaved (121°C, 1 atm, 30 min), followed by filtration through Whatman (100% cotton cellulose) filter paper to separate the soluble and insoluble ((Klason) fractions. Klason lignin (K) was calculated by the difference in the weight of the dry mass before and after filtration of each sample (Obst, Sachs, and Kuster 1988).

The acid soluble fraction (S) was determined spectrophotometrically, using ultraviolet absorption at 215 nm and 280 nm (JASCO V-630, USA), and calculated from the following formula:

$$S = (4.53A_{215} - A_{280})/300, \quad (1)$$

derived from the simultaneous resolution of two equations: $A_{280} = 0.68\ F + 18\ S$ and $A_{215} = 0.15\ F + 70\ S$, where A_{280} is the absorbance value at 280 nm, A_{215} is the absorbance value at 215 nm, F is the furfural concentration (g L^2), and S is the soluble lignin concentration (g L^2) (Moreira-Vilar et al. 2014). Total lignin content of feedstock material was estimated as the sum of K + S.

Quantification of crop output

Leaf crop: All edible jute leaves produced in each replicate were harvested on a day after flowering and weighed.

Grain crop: All rice grains were harvested after maturation; thereafter, each rice hill from each replicate was tagged, separately threshed, dried in the sun, and weighed.

Fruit crops: After the plants bore fruits, tomatoes and capsicum were harvested continuously, from all plots, for 22 days. The sum total of all the mature fruits from each plant counted and weighed after each harvest.

Root crop: Radish in both India and Thailand was harvested by uprooting the plants after the fruiting stage. Weights were taken after cleansing, washing, and drying in open air.

All weights reported here are fresh weights, taken after drying the materials in the air at ambient temperature, without using oven or desiccator.

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Statistical analyses

For each of the three treatments (A, B, and C in Table 3), we performed the following tests.

(1) a Kolmogorov–Smirnoff test for the normality assumption;
(2) a Q–Q plot in order to visualize the normality match of each of the three treatments; and
(3) a Levene test for the homogeneity of variance.
(4) Based on the positive outcome of tests 1 and 3, a one-way analysis of variance (ANOVA) (model 1) was performed. For the radish trial in Thailand, a two-way ANOVA (mixed model) was performed.
(5) Subsequently, a Student–Newman–Keuls (SNK) test was performed to identify appropriate subsets based on the proximity of means within the treatments.

Results

Yield results show wide variation between countries (Table 6). While the experiments with radish, rice, and tomato in India showed clear improvement of yield with Treatment A [biochar + PSM], compared to either Treatment B [biochar alone] or C [PSM alone], such distinctive differences were not prominent in the results of experiments conducted with jute in India, radish in Thailand and capsicum in the United Kingdom (Table 6). However, log-transformation of data enabled clear detection of significant crop yield enhancement in replicates treated with biochar and PSM. The results of statistical tests for each crop are described disparately below.

Table 6. Mean (all replications) and standard deviations of crop yield (g cm^{-2}).

	Treatments					
	(A) Biochar + PSM		(B) Biochar, no PSM		(C) PSM, no biochar	
Crop species	Mean	*SD*	Mean	*SD*	Mean	*SD*
Jute (India)	239.75	100.26	175.40	100.06	154.15	37.75
Rice (India)	182.08	22.36	136.67	6.08	157.50	16.96
Tomato (India)	1523.00	127.19	1258.20	138.61	1300.20	95.14
Capsicum (UK)	3137.78	1396.67	2354.59	1239.35	2241.33	1069.84
Radish (India)	908.00	310.99	361.80	136.52	517.15	169.15
Radish (Thailand)	1535.99	476.25	1141.09	694.73	806.61	176.83

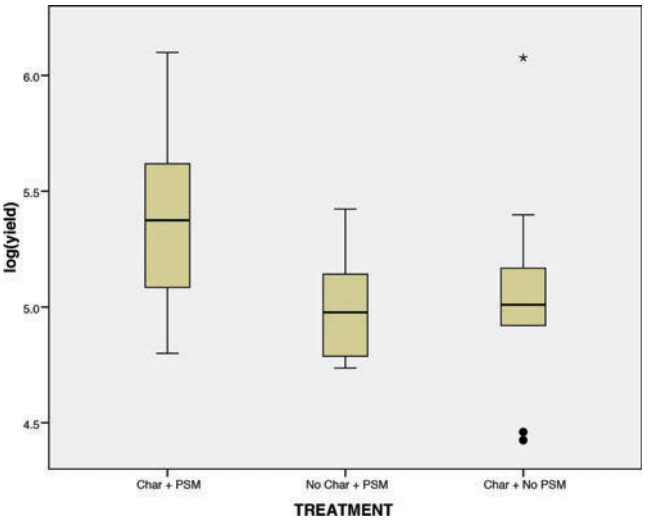

Figure 1. Box plot showing the range of log-transformed yields of jute leaf biomass in different treatments of biochar and PSM in India.

Jute (India): The mean of the yield of Treatment A (biochar and PSM) seems to be greater than that of both Treatments B and C. The difference appears to be prominent when the yield figures are log-transformed (Figure 1).

The KS test gave mixed results for jute data; while it confirmed the null hypothesis of normal distribution for both Treatments A and C, it rejected the null hypothesis for Treatment B (supplementary Table S1). Nevertheless, the Q–Q plot for Treatment B showed near normality of the distribution. After log-transformation of the data, however, the KS test showed normality of the data. The Levene test also rejected the null hypothesis that the noise associated with each treatment has identical variances (supplementary Table S2).

The yield from plots treated with biochar and PSM appear to be greater than other groups. The ANOVA of the log-transformed data showed marginally significant difference ($\alpha = 0.057$) among the treatments (Table 7). The

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Table 7. Result of one-way ANOVA of jute foliage yield (log transformed).

Treatment	SS	df	MS	F	α
Between groups	0.925	2	0.463	3.187	0.057
Within groups	3.919	27	0.145		
Total	4.844	29			

SNK test, however, detected difference of the Treatment A from other treatments only at the confidence level of α= 0.08 (supplementary Table S3).

Rice (India): The KS test failed to reject the null hypothesis of normality of the distribution (supplementary Table S4). The Levene test indicated a marginal violation of the null hypothesis that the noise associated with each treatment has identical variances (supplementary Table S5). Nevertheless, descriptive box plots clearly show the level of Treatment A to be well above the other two levels (Figure 2).

The ANOVA indicates a strong effect of the different treatments (Table 8). This result was corroborated by the SNK test, showing the mean yield of Treatment A constitutes a group distinctly different from both Treatments B and C (supplementary Table S6). The corresponding contrast test between the mean yield of group A and that of the combined (B + C) group is highly significant.

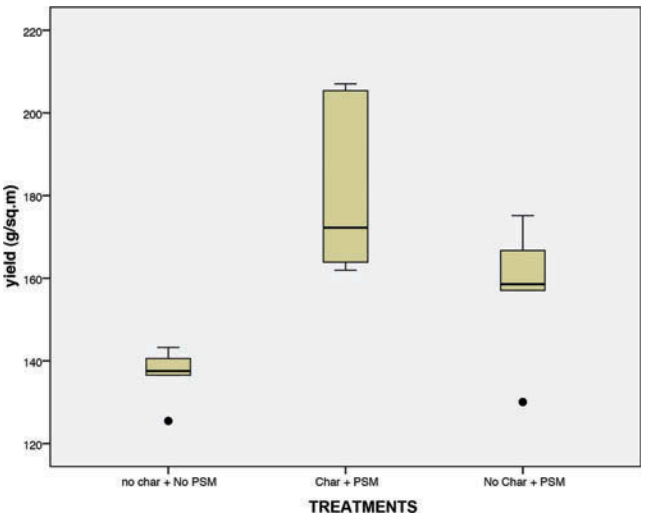

Figure 2. Box plot showing the range of rice yields in different treatments of biochar and PSM in India.

Table 8. Result of one-way ANOVA for rice grain yield.

Treatment	SS	df	MS	F	α
Between groups	5166.257	2	2583.129	9.293	0.004
Within groups	3335.629	12	277.969		
Total	8501.886	14			

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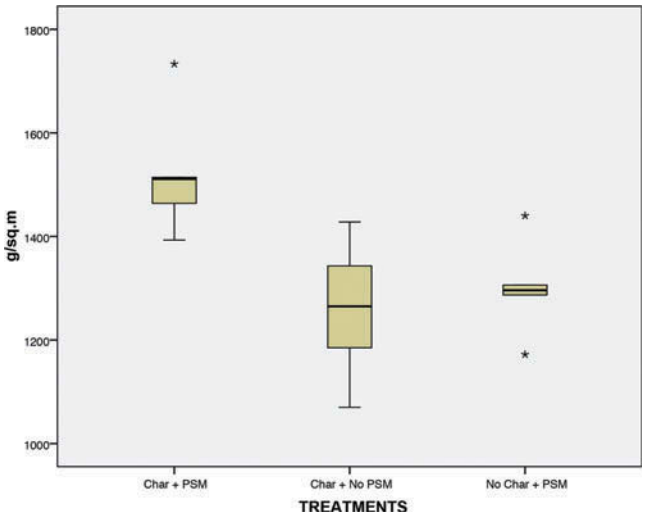

Figure 3. Box plot showing the range of tomato yields in different treatments of biochar and PSM in India.

Tomato (India): Mean fruit yield (g m^{-2}) in Treatment A appears to be significantly greater than that of both Treatments B and C (Figure 3).

The KS test validated the null hypothesis of normal distribution (supplementary Table S7) and the Levene test confirmed that the noise associated with each factor level has identical variances (supplementary Table S8). The positive results endorsed application of an ANOVA, which showed a highly significant ($F_{(df=2,12)}$ = 6.84) difference among the treatments (Table 9). The SNK test indicated that the mean yield of Treatment B (biochar, no PSM) and that of Treatment C (PSM, no biochar) may be grouped together, while Treatment A forms a disparate group (supplementary Table S9). The corresponding contrast test for the difference of the mean yield of Treatment A from that of the Treatments B and C combined is highly significant ($p < 0.005$)

Capsicum (UK): The ranges of capsicum yield appears to overlap between Treatments A, B, and C, although the mean yield for Treatment A appears to exceed that of both Treatments B and C (Figure 4).

The KS test indicated that the data for Treatment A do not conform to normal distribution, although the assumption of normality is not rejected for the other two Treatments B and C. In order to detect any plausible difference between the treatments at a higher resolution, we log_e-transformed the yield data, and

Table 9. Result of one-way ANOVA for tomato yield.

Treatment	SS	df	MS	F	α
Between groups	202538.133	2	101269.067	6.836	0.01
Within groups	177757.600	12	14813.133		
Total	380295.733	14			

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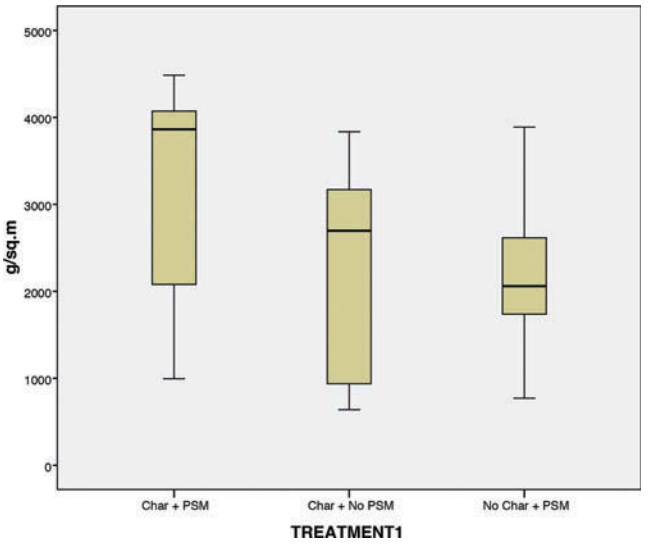

Figure 4. Box plot showing the range of capsicum yields in different treatments of biochar and PSM in the United Kingdom.

performed the KS test on the new data set (supplementary Table S10). The Levene test was unable to reject the null hypothesis that the noise associated with each factor level has identical variances (supplementary Table S11).

When the ANOVA was performed on the log_e-transformed yield data, no significant effect of the different treatments was detected (Table 10). The lack of statistical significance was corroborated by the SNK test (supplementary Table S12).

Radish (India): Mean yield of the root crop in Treatment A appears to exceed that of both Treatments B and C (Figure 5). However, the range of standard deviation for Treatment A is also wider than the other treatments.

The KS test and the Levene test validated the null hypothesis of normal distribution (supplementary Table S13) and of homogeneous variances (supplementary Table S14), respectively. The ANOVA shows a highly significant ($F_{(df\ =2,12)} = 7.775$, $\alpha = 0.007$) effect of the different treatments (Table 11). The SNK test further indicated that the mean yields of Treatments B and C together are significantly different from that of Treatment A.

Radish (Thailand): The range of yield estimates is wider for Treatment B than the other treatments, although the mean yield of the root crop appears to be greater for Treatment A than that of both Treatments B and C (Figure 6). In

Table 10. Result of one-way ANOVA for capsicum fruit yield (log-transformed).

	Sum of squares	df	Mean square	F	α
Between groups	0.642	2	0.321	0.829	0.448
Within groups	9.282	24	0.387		
Total	9.923	26			

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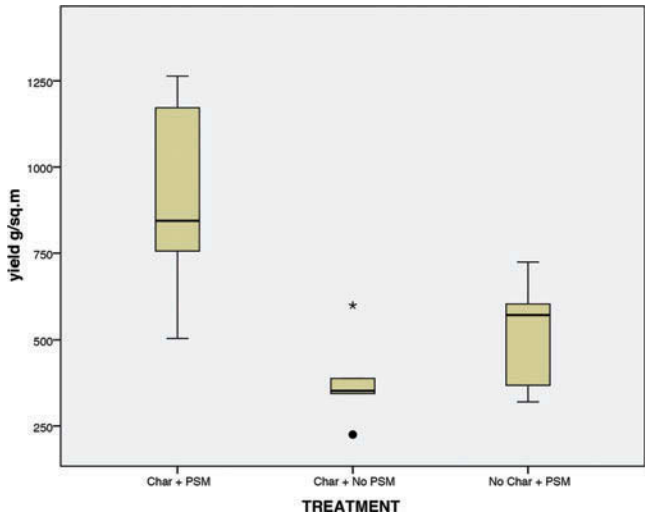

Figure 5. Box plot showing the range of radish yields in different treatments of biochar and PSM in India.

Table 11. Result of one-way ANOVA for radish yield in India.

Treatment	SS	df	MS	F	α
Between groups	746233.733	2	373116.867	7.775	0.007
Within groups	575852.00	12	47987.667		
Total	1322085.733	14			

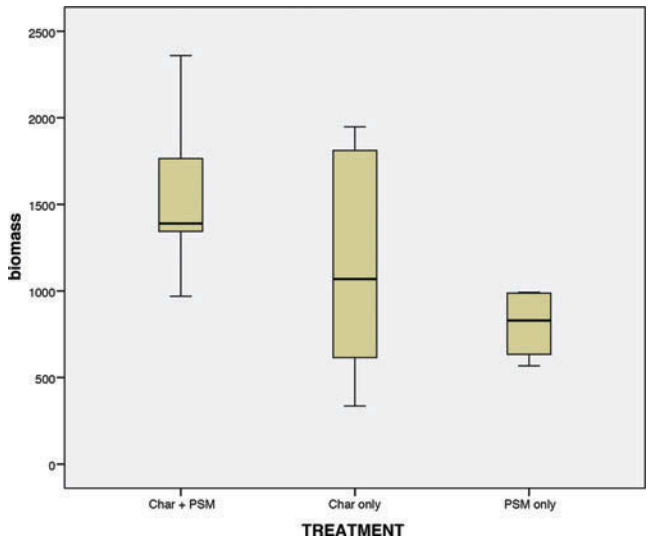

Figure 6. Box plot showing the range of radish yields in different treatments of biochar and PSM in Thailand.

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Table 12. Means and standard deviations of radish yield in different treatments of biochar, PSM, and soy manure in Thailand.

Treatment 1	Treatment 2	Mean	SD	N
Biochar + PSM	Soy	1822.66	510.14	3
	No soy	1249.33	245.44	3
	Total	1535.99	476.25	6
Biochar only, no PSM	Soy	1753.97	227.52	3
	No soy	528.22	167.31	3
	Total	1141.09	694.72	6
PSM only, no biochar	Soy	825.67	178.57	3
	No soy	787.55	212.77	3
	Total	806.61	176.91	6
Total	Soy	1467.44	564.39	9
	No soy	855.04	365.29	9
	Total	1161.24	558.54	18

this experiment, we added a second level of treatment, with the application of soy manure as an additional source of N. The differences in root crop biomass harvested from the two levels of treatment are summarized in Table 12.

The KS test was unable to reject the null hypotheses of normal distribution (supplementary Table S16). The Q–Q plot also shows near-normal distribution. However, the Levene test rejected the null hypothesis that the noise associated with each factor level has equal variances for Treatment A (supplementary Table S17).

Unequal variances for the treatments notwithstanding, we performed an ANOVA, for which we chose a very high significance level ($\alpha = 0.03$). The descriptive plot indicates significant differences between the components of Treatment 1. However, the one-way ANOVA results (Table 13) indicate low significance ($\alpha = 0.068$). Nevertheless, the SNK test shows that Treatments B and C form a set distinctly different from Treatment A. The difference between means of Treatment A and Treatments B and C combined, *with unequal variances*, is also significant ($\alpha = 0.04$).

When Treatment 2 is incorporated in the ANOVA, the results show significant differences in variance. The two-way ANOVA results indicate highly significant differences both within Treatment 1 ($\alpha = 0.003$) and Treatment 2 ($\alpha = 0.001$), although the interaction term (Treatment 1 × Treatment 2) is no significant (Table 14). The SNK test upholds separation of Treatment A from both Treatments B and C (supplementary Table S18). When Treatments B and C are clumped together and compared with Treatment A, discriminatory *t* test also shows highly significant difference of means between the two groups ($\alpha = 0.03$).

Table 13. Results of one-way ANOVA for radish yield in Thailand

Treatment	SS	df	MS	F	α
Between groups	1599673.492	2	799836.746	3.239	0.068
Within groups	3703777.957	15	246918.530		
Total	5303451.450	17			

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Table 14. Results of two-way ANOVA for radish yield in Thailand.

Treatment	Type III	df	MS	F	α
Corrected Model	4.349 x10^6	5	869726.914	10.931	0.000
Intercept	2.427 x10^7	1	2.427 × 10^7	305.053	0.000
Treatment 1	1599673.492	2	799836.746	10.052	0.003
Treatment 2	1687652.447	1	1687652.447	21.210	0.001
Treatment 1 × Treatment 2	1061308.630	2	530654.315	6.669	0.11
Error	954816.880	12	79568.073		

Discussion and conclusion

Our multilocation trials with biochar of different origins, indicate that biochar may have different effects on different types of crops, and that the effect on a particular crop may also vary at different soil quality parameters. In this study, biochar and PSM treatments showed a significant enhancing effect on the mean crop yield for jute, rice, radish, and tomato in India, and for radish in Thailand. By contrast, the biochar and PSM treatment seems to have little effect on the capsicum yield in the United Kingdom. In all these experiments, biochar alone had little beneficial effect on crop productivity.

The difference of the effects of biochar application in different crop experiments cannot be attributed to the different feedstock sources of biochar used in our study. Since all biochar material was prepared at all three sites by slow pyrolysis of lignin-rich wood at low temperature, the biochar type remained essentially the same, and, therefore, is unlikely to elicit any significant difference in the physical and sorptive properties of the biochar particles. Furthermore, the pyrolysis temperature at all three sites ranged between 350 and 500°C, and therefore, may not cause any difference in C sequestration balances (Lehman, Gaunt, and Rondon 2006; Yargicoglu et al. 2015).

Results of this study indicate that biochar/PSM synergy may have significant positive effects on the yield of grain crops and root crops, but not significantly for leaf crops. Furthermore, yield-enhancing effect of biochar varies between the fruit crops (tomato in India and capsicum in the United Kingdom). Therefore, soil characteristics and crop type are more likely to determine the impact of biochar on specific crop output than could the feedstock species. This is corroborated by the difference in radish output in India and in Thailand. The two-factor input experiment with radish in Thailand further shows that input regimes, especially the levels of N and P inputs make crucial differences.

As the rate of nutrient flow depends crucially on the soil type, "not all soils will benefit from biochar applications" (Ippolito, Laird, and Busscher 2012:971). Biochar treatments may improve soil quality or release of nutrients to plants, but the addition of biochar is likely to yield greater benefits to degraded, sandy, or nutrient-poor soils than to highly fertile soils (Sohi et al. 2010; Ippolito, Laird, and Busscher 2012). Biochar application to nutrient-

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poor soils with low pH (e.g., tropical oxisol) is recorded to have improved crop yields (Rondon et al. 2007; Major et al. 2010). Our study seems to corroborate this observation: The predominance of silt, and higher P content in the soils of Thailand and UK farms, seems to have responded less to biochar amendment than did the tropical oxisol of the Indian farm.

Soil inorganic P seems to be more important than N in biochar-treated soils, because biochar can inhibit the nitrification process or immobilize previously plant available N, and thereby reduce plant N uptake (Warnock et al. 2007; Sohi et al. 2010; Clough et al. 2013), whereas available P is generally increased upon the incorporation of PSM in biochar (Zheng, Sharma, and Rajagopalan 2010; Karer et al. 2013). High P content of the soil seems to delimit the beneficial effect of biochar: the combined application of biochar and PSM does not improve crop productivity grown in the soil already endowed with very high P (as in the UK glasshouse experiment). Crop productivity is more enhanced by biochar and PSM application in P-impoverished soils (as in the Indian farm soil) than in soils with higher levels of P (as in the Thailand farm and the UK greenhouse soil).

Recent research indicates that many plants have several types of physiological adaptation to P-starvation, including enhanced uptake ability through activation of high affinity transporters and adaptive root development, induction of phosphate scavenging and recycling enzymes, etc. (e.g., Vance, Uhde-Stone, and Allan 2003). In rice, for example, the OSPTF1 (*Oryza sativa* phosphate transcription factor) gene plays a vital role in the increased tolerance to P-deficiency in soil. Phosphoenolpyruvate (PEP) plays a central role in the modification of carbon and energy metabolism in response to P-starvation. In the cytosol, PEP can be converted to pyruvate catalyzed by pyruvate kinase or to oxaloacetate catalyzed by PEP carboxylase, which is suggested to be a P-starvation-induced bypass to preserve P in cytosol (Hou et al. 2005). Biochar-induced increase in PSM community, and their activities in P-deficient soils, serves to reduce soil P deficit, and facilitates residual P uptake by plant roots.

Conversely, in soils that are already rich in available P, the presence or addition of PSM is unlikely to mobilize more soluble phosphates. Rather, an excess of P might plausibly elicit a negative feedback on PSM activity, in order to maintain an equilibrium level of P in the flux. Johnston's (2005) experiment on the P uptake at different levels of P shows conclusively that the P uptake and yields of sugar beet, barley and winter wheat grown on different soils at three sites in the southeast of the United Kingdom reaches an asymptote at higher levels of available Olsen P. This study showed that above certain critical value of P in soil, there was no further increase in crop yield with further increases in available P. For each of the three crops, the Olsen P value at which the yield approached the asymptote was similar irrespective of the large annual differences in yield. This P uptake dynamics

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may be described as a positive nonlinear function of P uptake (U) with available P level in soil,

$$U = f(C_P), \tag{2}$$

where C_P is the concentration of available P in soil. The rate of P uptake increases with C_P until reaching a critical upper limit for P uptake (C_k):

$$U' = r\,C_P(1 - C_P/C_k) \text{ for } 0 < C_P < C_k \tag{3a}$$

$$U' = 0 \text{ for } C_P > C_k, \tag{3b}$$

where $U' = \delta U/\delta\, C_P$ is the marginal P uptake with respect to available P concentration in soil, and r is the intrinsic rate of P uptake by plants. At available P concentrations above C_k the differential uptake rate $\hat{U} = 0$, so the actual P uptake by plants does not increase. Figure 7 plots this general P uptake dynamics of Equations (3a) and (3b), with an empirically estimated C_k of around 8 mg kg^{-1} (Johnston 2005).

Review of literature depicting the influence of available P in soil on crop yield (Syers, Johnston, and Curtin 2008) indicates that above the "critical level" of available P in soil, any additional supply of P "is inefficient, because there is no increase in yield" (Syers, Johnston, and Curtin 2008:40). (The Figure 5 and Figure 6 in Syers, Johnston, and Curtin (2008) are an exact presentation of our Equation (2).) Thus, in sufficiently P-rich soil, further addition of biochar or PSM or P fertilizer would have little effect to enhance PSM growth, nor enhancement in P uptake, and consequently, no improvement in plant productivity. This is demonstrated in the UK capsicum experiment with very high soil P content (no yield advantage), and in the Thai radish experiment, with moderate level of P in soil, in contrast with the nutrient-poor oxisol in India, where yield enhancement was prominent.

The results of our study, in conformity with the findings reported in Syers, Johnston, and Curtin (2008), seem to support the above conjecture depicted

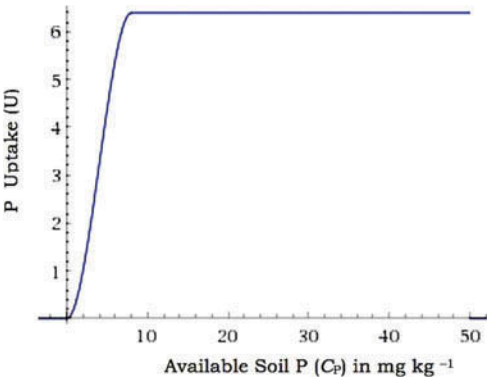

Figure 7. Plot of Equation (3) showing no increase in P uptake beyond C_K = 8 mg kg^{-1}.

in Figure 8. Biochar serves to foster growth and activities of PSM, which would increase P availability to plants. Contrariwise, when either PSM or biochar is absent, no increase in P bioavailability can be detected. This is evidenced in our experiments with most of the crops, where the effect of Treatment A (biochar + PSM) is significantly positive, compared to both treatments B (biochar alone) and C (PSM alone). However, when the soil has sufficiently high levels of P, the presence of PSM and/or biochar would have no significant effect on crop yield.

To test the general validity of our schema of biochar/PSM synergistic effect on crop yield, a meta-analysis of a wide range of biochar experiments conducted in soils of different qualities, is warranted. In the absence

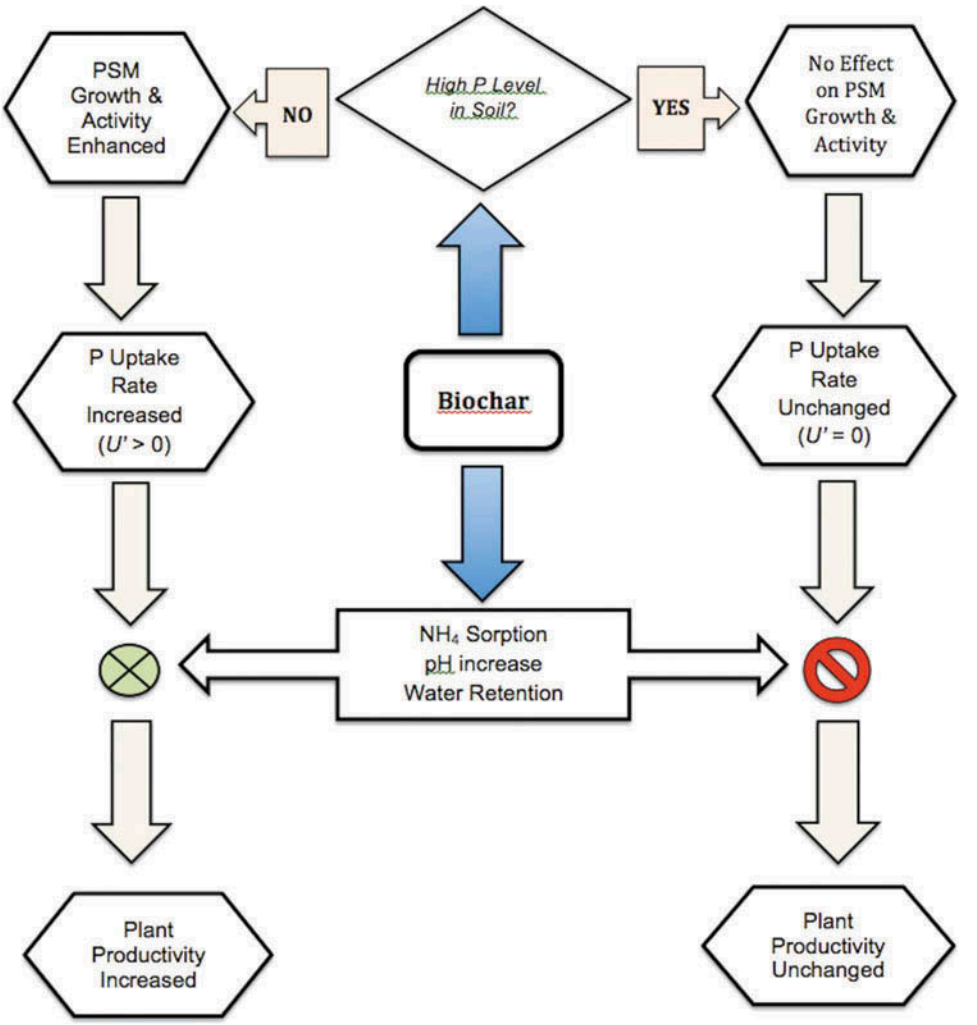

Figure 8. A schema of crop yield-enhancing effect of biochar in P-deficient soil. (*Summing junction*: synergistic combination of processes. *"No" symbol*: Absence of effect).

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of such meta-analysis, further experimental evidence is needed to clarify the relationship between biochar quality, soil nutrient levels, and crop types.

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Supplemental material

Supplemental material for this article can be found on the publisher's website.

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SUPPLEMENTARY MATERIALS

1. TABLES

Table S1: Kolmogorov-Smirnov Test of Normality for Jute Foliage Yield (log-Transformed) in Three Treatments.

Treatment	**KS Statistic***	***df***	**α**
A (Biochar + PSM)	0.192	10	0.20
B (Biochar, No PSM)	0.207	10	0.20
C (PSM, No Biochar)	0.138	10	0.20

* Lilliefors significance correction

Table S2: Levene Test of Homogeneity of Variance for Jute Foliage Yield (log-Transformed) in Three Treatments.

Yield Data	Levene Statistic	*df*1	*df*2	α
Based on Mean	1.27	2	27	0.29
Based on Median	1.29	2	27	0.29
Based on Median and with adjusted *df*	1.29	2	18.05	0.29
Based on trimmed Mean	1.28	2	27	0.29

Table S3: Student-Newman-Keuls Test of Homogeneous Subsets for Jute Yield (log-Transformed) in Three Treatments.

Treatment	***N***	**Means for Groups in Homogeneous Subsets**
		1
A	10	5.405
B	10	5.057
C	10	5.013
α		0.072

Table S4: Kolmogorov-Smirnov Test of Normality for Rice Grain Yield in Three Treatments in India.

Treatment	**KS Statistic**	***df***	***P***
A (Biochar + PSM)	0.292	5	0.19
B (Biochar, No PSM)	0.270	5	0.20
C (PSM, No Biochar)	0.289	5	0.19

Table S5: Levene Test of Homogeneity of Variance for Rice Grain Yield in Three Treatments in India.

Yield Data	Levene Statistic	*df*1	*df*2	α
Based on Mean	4.37	2	12	0.04
Based on Median	1.54	2	12	0.25
Based on Median and with adjusted *df*	1.54	2	8.29	0.27
Based on trimmed Mean	4.22	2	12	0.041

Table S6: Student-Newman-Keuls Test of Homogeneous Subsets for Rice Grain Yield in Three Treatments in India.

Treatment	*N*	Means for Groups in Homogeneous Subsets	
		1	2
A	5		182.08
B	5	136.67	
C	5	157.50	
α		0.072	1.00

Table S7: Kolmogorov-Smirnov Test of Normality for Tomato Yield in Three Treatments in India.

Treatment	KS Statistic*	*df*	α
A (Biochar + PSM)	0.328	5	0.08
B (Biochar, No PSM)	0.130	5	0.20
C (PSM, No Biochar)	0.276	5	0.20

Table S8: Levene Test of Homogeneity of Variance for Tomato Yield in Three Treatments in India.

Yield Data	Levene Statistic	*df*1	*df*2	α
Based on Mean	0.456	2	12	0.64
Based on Median	0.401	2	12	0.68
Based on Median and with adjusted *df*	0.401	2	11.32	0.68
Based on trimmed Mean	0.437	2	12	0.66

Table S9: Student-Newman-Keuls Test of Homogeneous Subsets for Tomato Yield in Three Treatments in India.

Treatment	*N*	Means for Groups in Homogeneous Subsets	
		1	**2**
A	5		1523.00
B	5	1258.20	
C	5	1300.20	
α		0.59	1.00

Table S10: Kolmogorov-Smirnov Test of Normality for Capsicum Yield in Three Treatments in the UK.

Treatment	KS Statistic*	*df*	α
A (Biochar + PSM)	0.254	9	0.09
B (Biochar, No PSM)	0.207	9	0.20
C (PSM, No Biochar)	0.141	9	0.20
Treatment (log-Transformed)			
A (Biochar + PSM)	0.306	9	0.15
B (Biochar, No PSM)	0.278	9	0.43
C (PSM, No Biochar)	0.179	9	0.20

Table S11: Levene Test of Homogeneity of Variance for Capsicum Yield (log-Transformed) in Three Treatments in the UK.

Yield Data	Levene Statistic	*df*1	*df*2	α
Based on Mean	0.998	2	24	0.38
Based on Median	0.177	2	24	0.84
Based on Median and with adjusted *df*	0.177	2	21.41	0.84
Based on trimmed Mean	0.860	2	24	0.44

Table S12: Student-Newman-Keuls Test of Homogeneous Subsets for Capsicum Yield (log-Transformed) in Three Treatments in the UK.

Treatment	*N*	Means for Groups in Homogeneous Subsets
		1
A	9	7.58
B	9	7.59
C	9	7.91
α		0.50

Table S13: Kolmogorov-Smirnov Test of Normality for Radish Yield in Three Treatments in India.

Treatment	**KS Statistic***	***df***	**α**
A (Biochar + PSM)	0.202	5	0.20
B (Biochar, No PSM)	0.282	5	0.20
C (PSM, No Biochar)	0.226	5	0.20

* Lilliefors significance correction

Table S14: Levene Test of Homogeneity of Variance for Radish Yield in Three Treatments in India.

Yield Data	Levene Statistic	*df*1	*df*2	α
Based on Mean	2.99	2	12	0.88
Based on Median	1.64	2	12	0.23
Based on Median and with adjusted *df*	1.64	2	9.27	0.25
Based on trimmed Mean	3.05	2	12	0.08

Table S15: Student-Newman-Keuls Test of Homogeneous Subsets for Radish Yield in Three Treatments in India.

Treatment	***N***	**Means for Groups in Homogeneous Subsets**	
		1	**2**
A	5		908.00
B	5	381.80	
C	5	517.60	
α		0.35	1.00

Table S16: Kolmogorov-Smirnov Test of Normality for Radish Yield in Three Treatments in Thailand.

Treatment	**KS Statistic***	***df***	**α**
A (Biochar + PSM)	0.257	6	0.20
B (Biochar, No PSM)	0.267	6	0.20
C (PSM, No Biochar)	0.180	6	0.20

* Lilliefors significance correction

Table S17: Levene Test of Homogeneity of Variance for Radish Yield in Three Treatments in Thailand.

Yield Data	Levene Statistic	*df*1	*df*2	α
Based on Mean	8.51	2	15	0.003
Based on Median	5.72	2	15	0.014
Based on Median and with adjusted *df*	5.72	2	8.42	0.027
Based on trimmed Mean	8.23	2	15	0.004

Table S18: Student-Newman-Keuls Test of Homogeneous Subsets for Radish Yield in Three Treatments in Thailand.

Treatment	***N***	**Means for Groups in Homogeneous Subsets**
		1
A	6	1535.99
B	6	1141.099
C	6	806.61
α		0.055

2. FIGURES

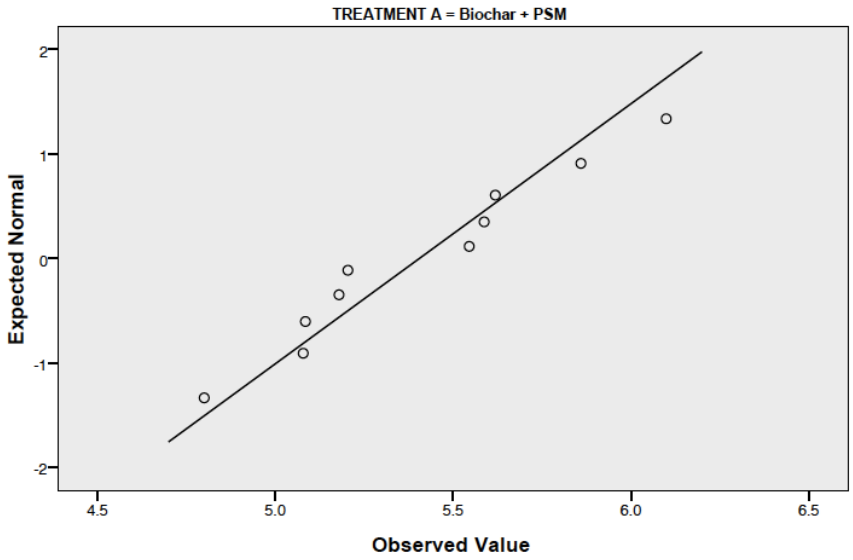

Fig. S1-A: Q-Q Plot of the Edible Jute Yield for Treatment A in India.

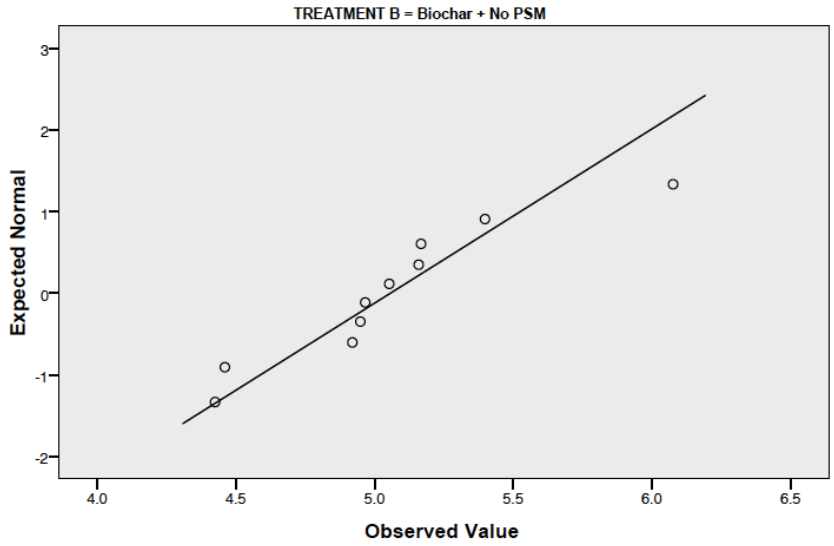

Fig. S1-B: Q-Q Plot of the Edible Jute Yield for Treatment B in India.

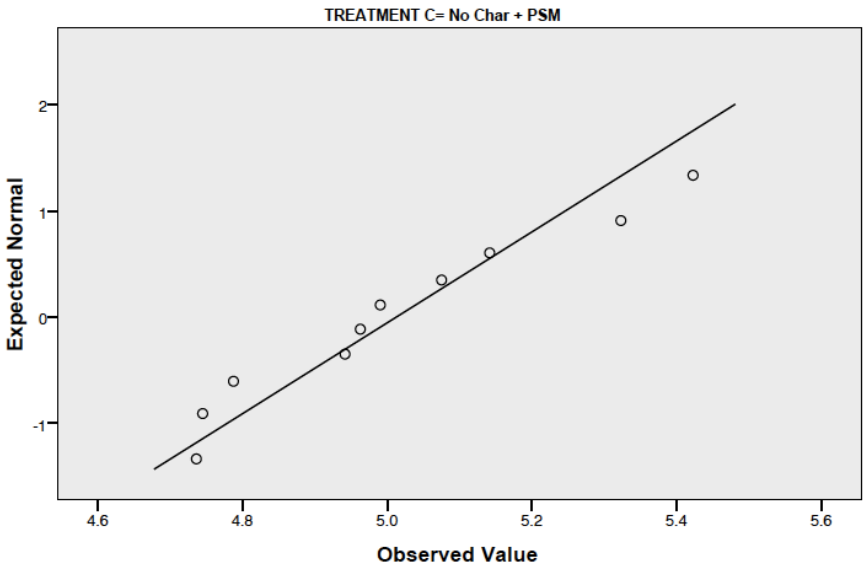

Fig. S1-C: Q-Q Plot of the Edible Jute Yield for Treatment C in India.

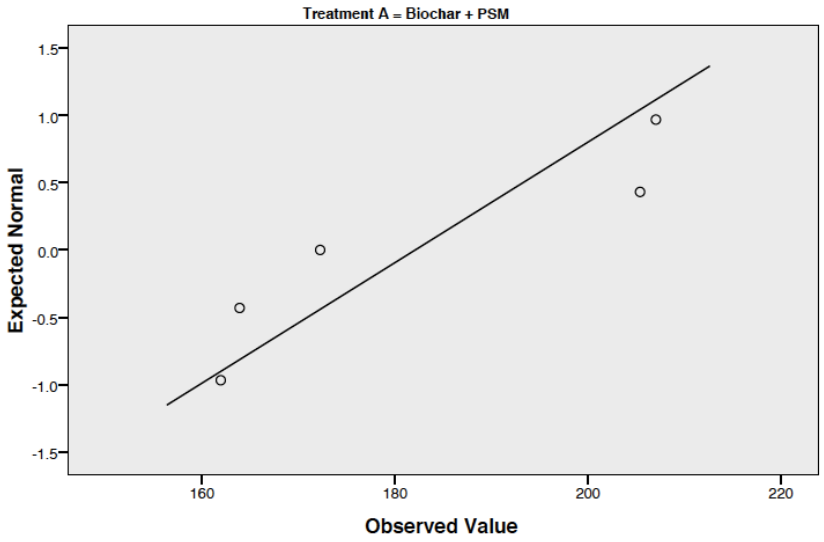

Fig. S2-A: Q-Q Plot of Rice Grain Yield for Treatment A in India.

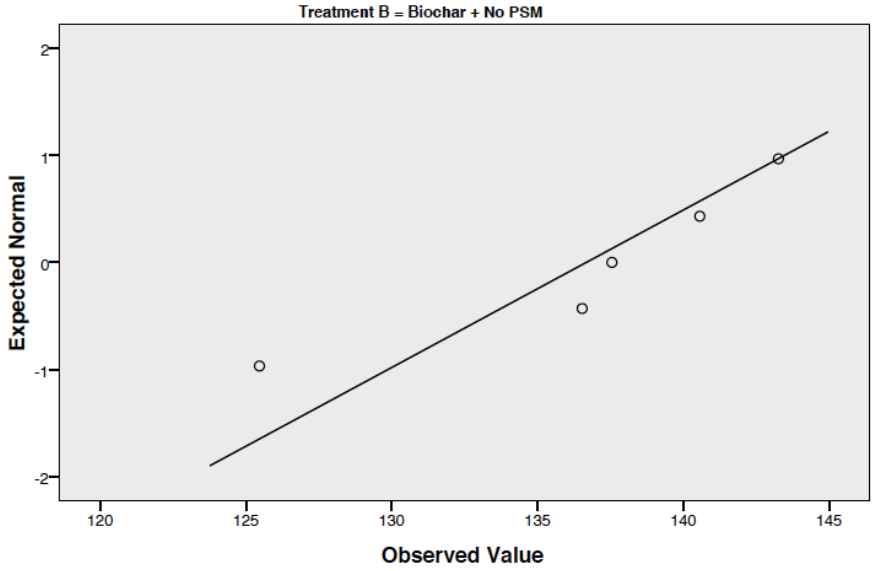

Fig. S2-B: Q-Q Plot of Rice Grain Yield for Treatment B in India.

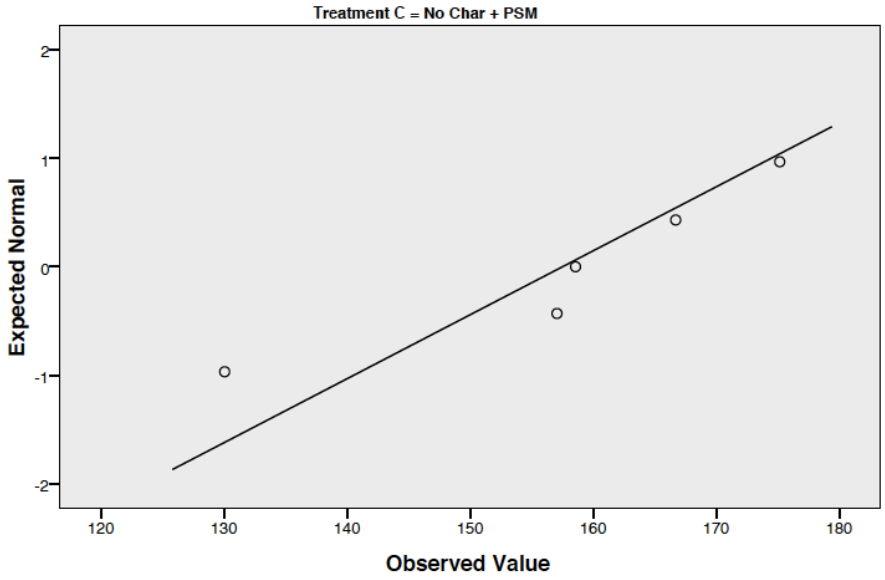

Fig. S2-C: Q-Q Plot of Rice Grain Yield for Treatment C in India.

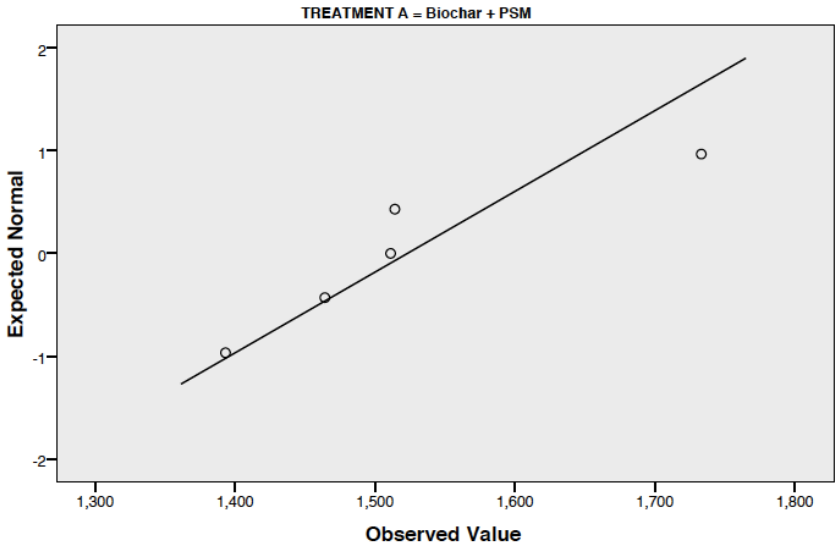

Fig. S3-A: Q-Q Plot of Tomato Yield for Treatment A in India.

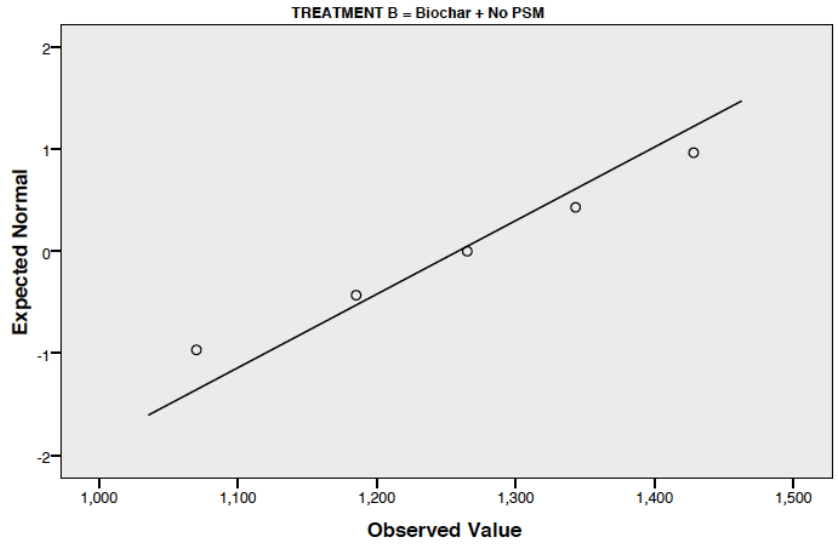

Fig. S3-B: Q-Q Plot of Tomato Yield for Treatment B in India.

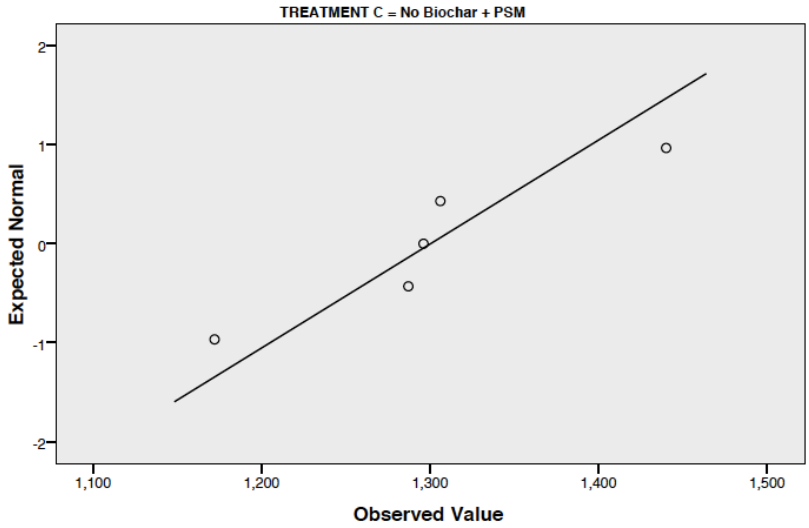

Fig. S3-C: Q-Q Plot of Tomato Yield for Treatment C in India.

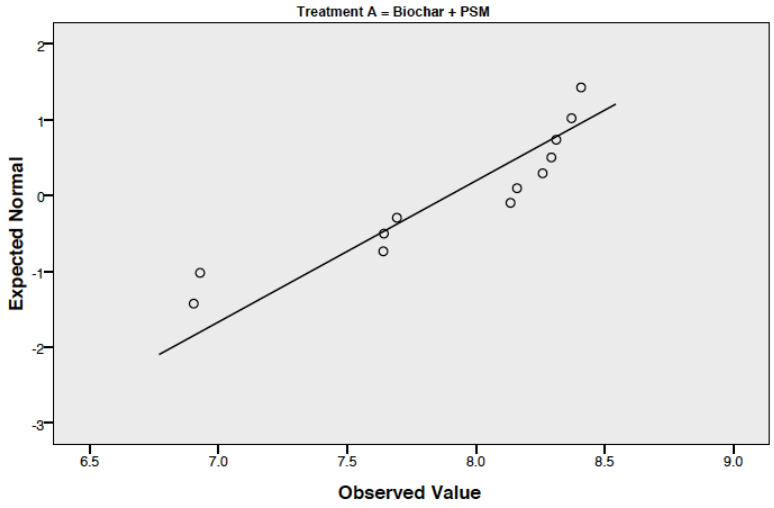

Fig. S4-A: Q-Q Plot of Capsicum Yield for Treatment C in the UK.

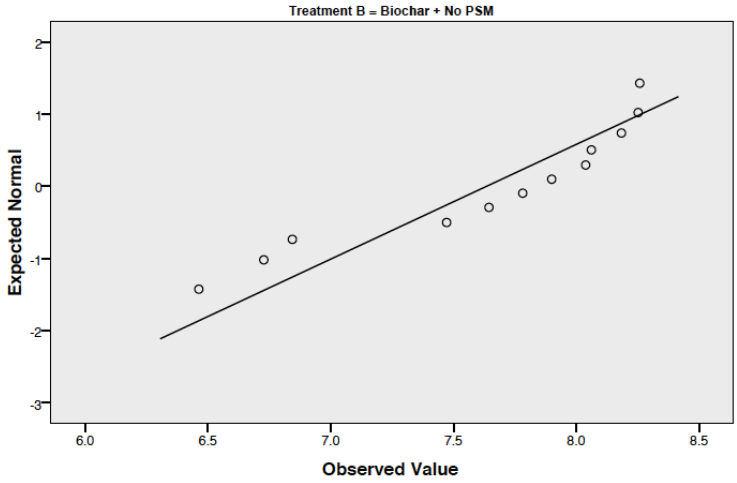

Fig. S4-B: Q-Q Plot of Capsicum Yield for Treatment C in the UK.

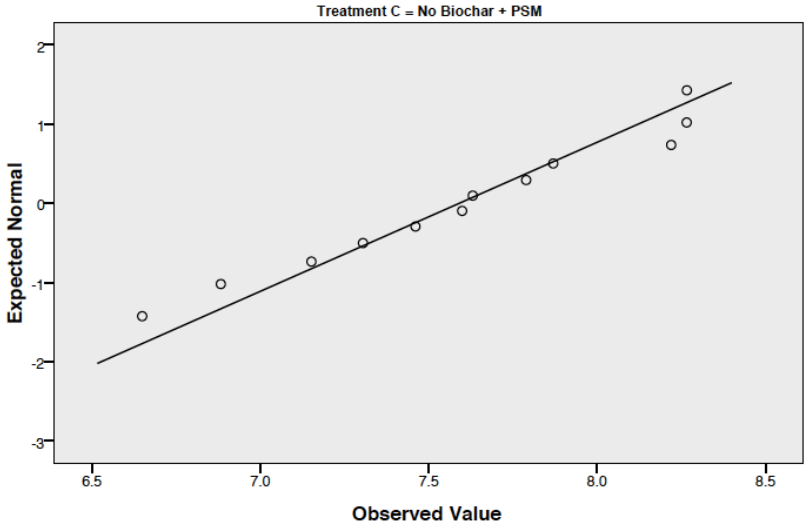

Fig. S4-C: Q-Q Plot of Capsicum Yield for Treatment C in the UK.

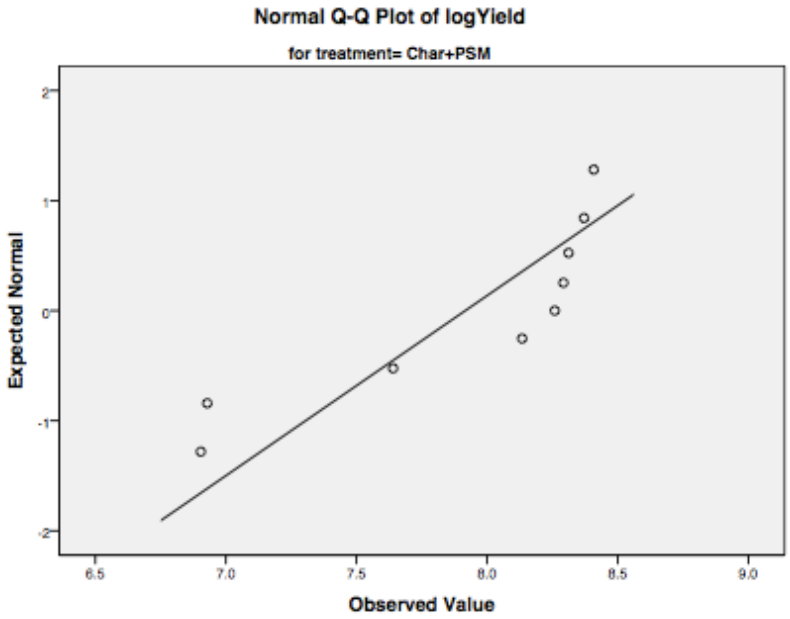

Fig. S5-A: Q-Q Plot of Capsicum Yield for Treatment A in the UK.

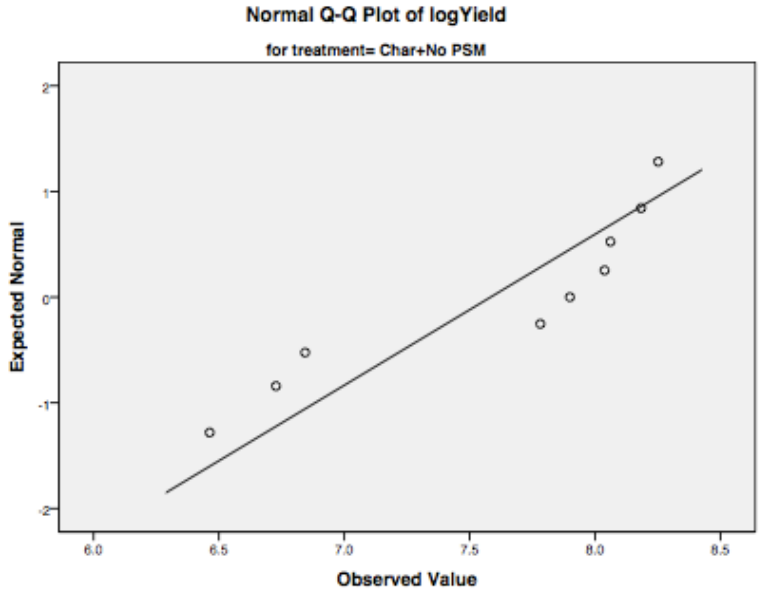

Fig. S5-B: Q-Q Plot of Capsicum Yield for Treatment B in the UK.

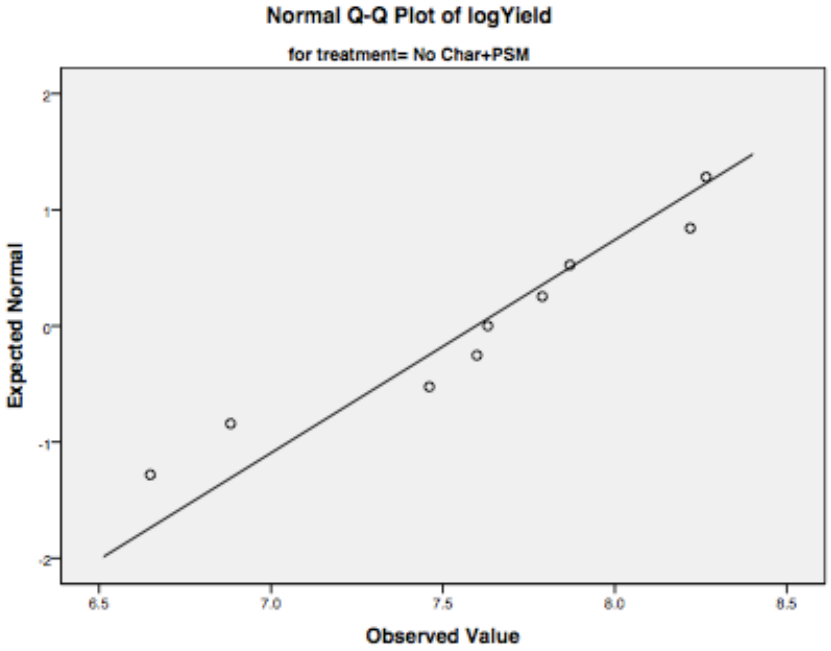

Fig. S5-C: Q-Q Plot of Capsicum Yield for Treatment C in the UK.

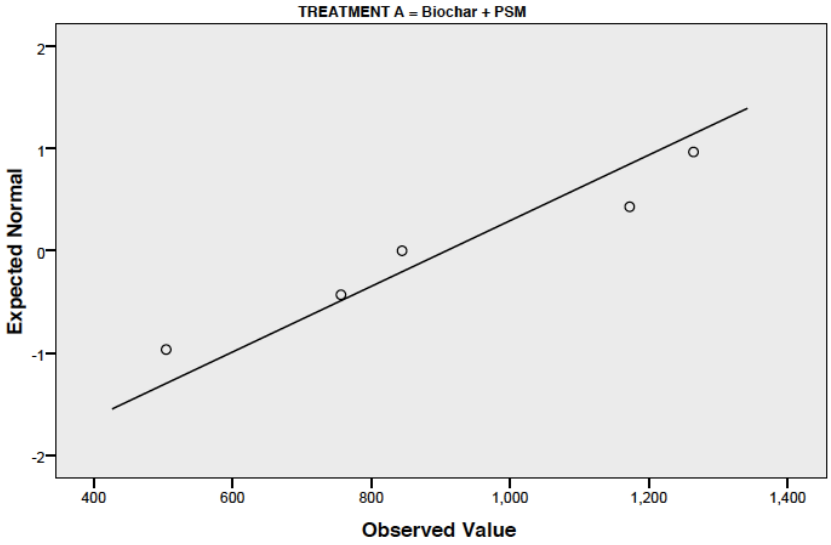

Fig. S6-A: Q-Q Plot of Radish Yield for Treatment A in India.

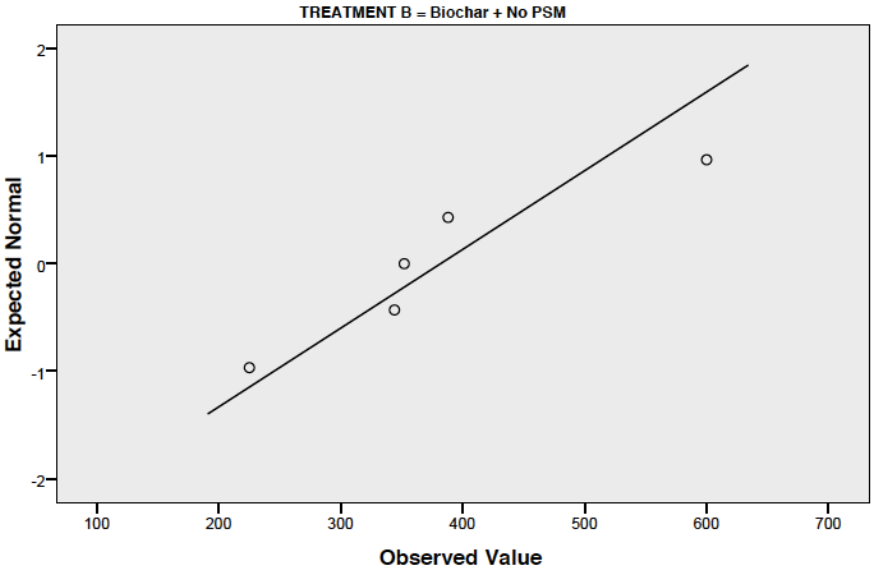

Fig. S6-B: Q-Q Plot of Radish Yield for Treatment B in India.

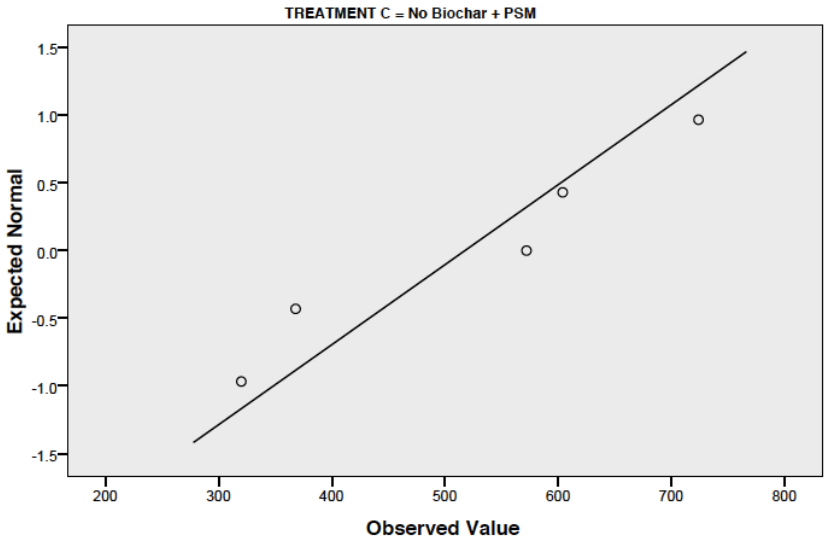

Fig. S6-C: Q-Q Plot of Radish Yield for Treatment C in India.

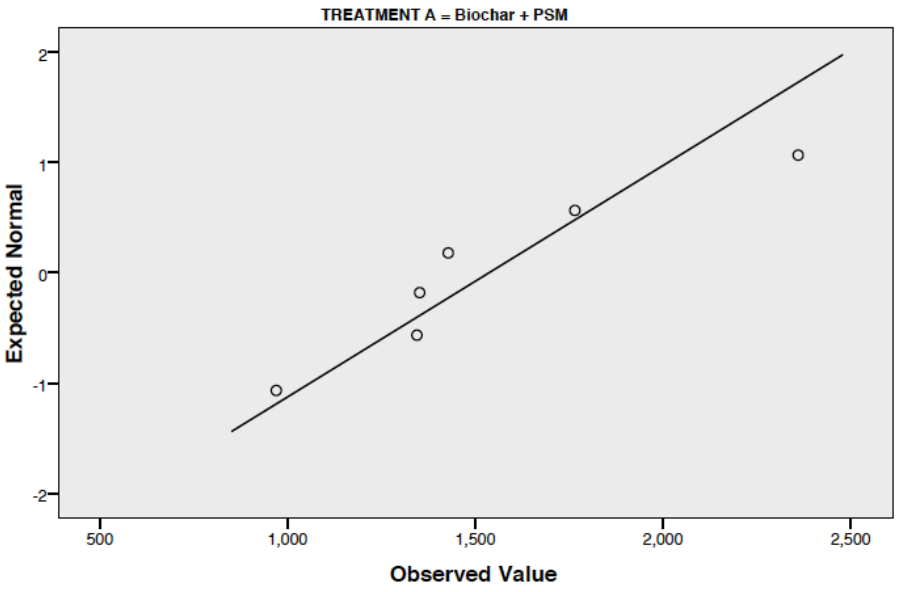

Fig. S7-A: Q-Q Plot of Radish Yield for Treatment A in Thailand.

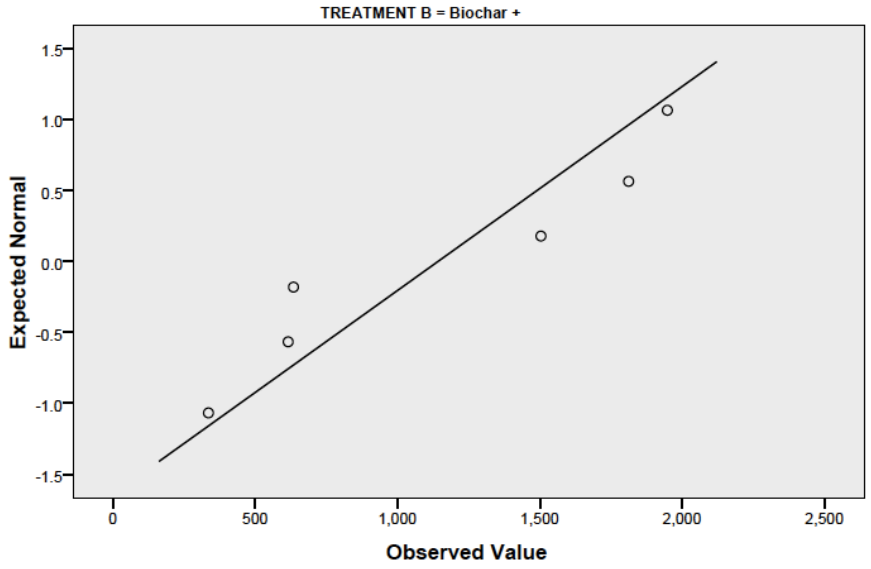

Fig. S7-B: Q-Q Plot of Radish Yield for Treatment B in Thailand.

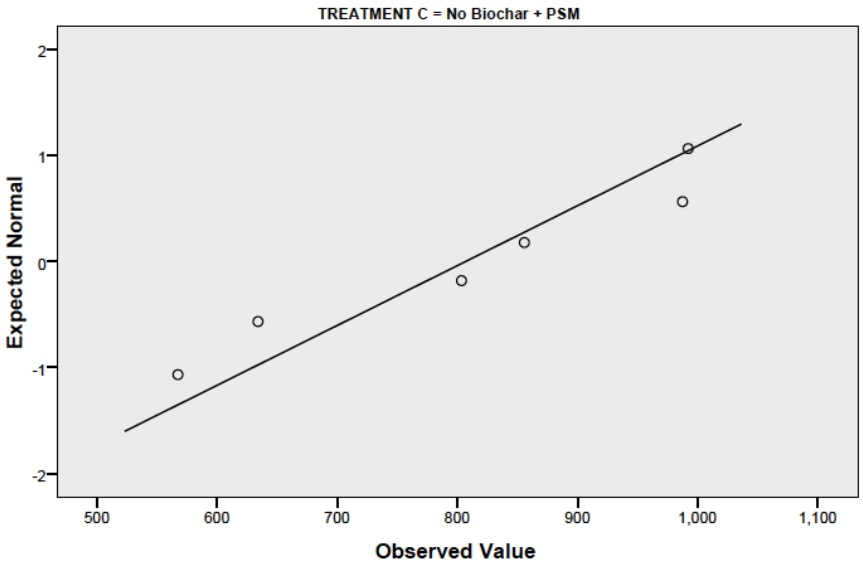

Fig. S7-C: Q-Q Plot of Radish Yield for Treatment C in Thailand.