agronomy

MDPI

Article

Fertility Impact of Separate and Combined Treatments with Biochar, Sewage Sludge Compost and Bacterial Inocula on Acidic Sandy Soil

Nikolett Uzinger [1,*], Tünde Takács [1], Tibor Szili-Kovács [1], László Radimszky [1], Anna Füzy [1], Eszter Draskovits [1], Nóra Szűcs-Vásárhelyi [1], Mónika Molnár [2], Éva Farkas [2], József Kutasi [3] and Márk Rékási [1]

1 Institute for Soil Sciences and Agricultural Chemistry, Centre for Agricultural Research, Herman O. út 15., 1022 Budapest, Hungary; takacs.tunde@atk.hu (T.T.); szili-kovacs.tibor@atk.hu (T.S.-K.); radimszky.laszlo@atk.hu (L.R.); fuzy.anna@atk.hu (A.F.); draskovits.eszter@atk.hu (E.D.); szucs-vasarhelyi.nora@atk.hu (N.S.-V.); rekasi.mark@atk.hu (M.R.)

2 Department of Applied Biotechnology and Food Science, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Műegyetem rkp. 3., H-1111 Budapest, Hungary; molnar.monika5@gmail.com (M.M.); eva.farkas22@gmail.com (É.F.)

3 BioFil Microbiological, Gene-Technological and Biochemical Ltd., Váci út 87., 1139 Budapest, Hungary; biokutasi@gmail.com

* Correspondence: uzinger.nikolett@atk.hu

Received: 25 August 2020; Accepted: 19 October 2020; Published: 21 October 2020

check for updates

Abstract: The short-term effects of processed waste materials: sewage sludge compost (up to 0.5%), biochar made of paper sludge and grain husk (BC) (up to 2%) combined with plant growth-promoting rhizobacterial (PGPR) inoculum, on the fertility of acidic sandy soil at 65% of field capacity were tested in a pot experiment in separate and combined treatments. The soil pH, organic matter content, total and plant-available nutrients, substrate-induced respiration, arbuscular mycorrhizal fungal (AMF) root colonisation parameters and maize (*Zea mays* L.) biomass were investigated in experiments lasting two months. The positive priming (21% organic matter loss) induced by BC alone was not observed after combined application. The combination of compost and PGPR with 1.5% BC resulted in 35% higher P and K availability due to greater microbial activity compared to BC alone. Only compost applied alone at 0.5% gave a 2.7 times increase in maize biomass. The highest microbial activity and lowest AMF colonisation were found in combined treatments. In the short term the combined application of BC, compost and PGPR did not result in higher fertility on the investigated soil. Further research is needed with a wider range of combined treatments on acidic sandy soil for better understanding of the process.

Keywords: fertilisation; soil amelioration; *Zea mays*; waste reutilisation; PGPR

1. Introduction

On sandy soils crop production is limited by several factors, the most important of which are low water retention capacity and nutrient content [1]. Due to the texture of these soils, organic matter (OM) is mineralised at a higher rate, leading to reduced fertility [2]. Fertilisation is less effective on these soils, as the nutrients added with mineral fertilisers have low colloid content and are easily leached [3]. On such soils irrigation and/or soil amelioration are prerequisites for safe cultivation, especially in the case of less drought-resistant crops like maize.

One possible way of improving such degraded or inherently unfavourable soils is to incorporate organic materials. Composts could be useful amendments for sandy soil, as they may increase the

OM content of the soil, improve the soil water capacity and aggregation stability, and increase the cation exchange capacity, and they can also be used as fertilisers [1,4]. The OM from composts has a significant influence on the status of the soil microbial biomass, as it provides a new energy source [5]. However, these effects may be short-lived in sandy soils due to the fast mineralisation of OM.

A longer term ameliorating effect can be achieved by the application of biochar (BC), which is able to improve the physical and hydrological properties and buffering capacity of the soil. BC may also contain a significant amount of humic acid, which could have a positive effect on the physicochemical properties of the soil [6]. Furthermore, BC influences the abundance and community composition of soil microbes, including arbuscular mycorrhizal fungi (AMF), though the published findings are contradictory [7,8].

BC is a recalcitrant material, resistant to biodegradation and chemical decomposition. Depending on the pyrolysis conditions and feedstock, it may contain a labile OM fraction, but this may be mineralised in the soil within a month or two of application [9], thus reducing the amount of OM available. In addition, due to the high C/N ratio of BC, this mineralisation may also cause the positive priming of the native organic matter in the soil, which has an adverse effect in the long term [10].

The mineralisation of the labile fraction also involves the release of nutrients, which, together with the soluble inorganic nutrient content of BC, contributes to its fertiliser effect. However, due to its adsorption capacity, BC may also decrease the mobility of nutrients and their uptake by plants [11]. Contradictory results have therefore been obtained regarding the fertiliser effect of BC [12]. To avoid a decrease in the availability of nutrients, it is advisable to use BC in combination with fertiliser [13,14].

The positive priming and reduced nutrient availability that may be observed after BC application can be mitigated by applying an organic fertiliser such as compost. Composts containing sewage sludge are very appropriate for agricultural utilisation such as fertilisation and soil conditioning due to their high nitrogen and phosphorus content and organic matter [15]. The availability of the nutrients in these composts may be moderated by adsorption on BC, making the nutrient content more evenly available for a longer period [16]. BC may protect the labile OM of compost from mineralisation, which could again contribute to a balanced nutrient supply [17].

Nutrient availability after BC application can be further improved using plant growth-promoting rhizobacteria (PGPR), which represent an environmental-friendly way of improving the fertility of soils. These bacteria are able to fix nitrogen, solubilise phosphorus, sequester iron, produce plant growth hormones, antibiotics and antifungal compounds, and enhance the competitive exclusion of plant pathogens [18].

The effect of combining organic amendments with PGPR on soil fertility and other soil services has already been tested, but data on the joint application and interactive effect of compost, BC and PGPR are scarce [8,15,19,20]. The combined application of these materials proved successful in alleviating drought stress and in phytoremedial technologies [4,21,22]. The question raised in the present research was whether the use of compost and selected PGPRs was able to improve the fertility of a BC-amended acidic sandy soil with adequate water supplies, by balancing the biological and physicochemical properties and improving the availability of nutrients.

The objective of the study was thus to test whether the interactive effect of BC, sewage sludge compost and PGPR on soil OM and nutrient supply was more favourable than the separate effects of these materials. It was hypothesised that, when jointly applied, sewage sludge compost, PGPR and BC would complement each other synergistically, resulting in better agronomical effects than the separate treatments. A decrease in the mineralisation of added OM, an increase in microbial activity and higher macronutrient availability were expected after combined application, resulting in higher maize biomass.

2. Materials and Methods

2.1. Materials

The acidic sandy soil used in the pot experiment was taken from the ploughed layer (0–20 cm) of a field at the experimental station of the Institute for Soil Sciences and Agricultural Chemistry, Centre for Agricultural Research, in Nyírlugos, Hungary (47°43′ N, 22°00′ E). The particle size distribution of the soil was >0.05 mm: 85%, 0.05–0.002 mm: 10%, <0.002 mm: 5%. The BC applied was selected based on the evaluation of Molnár et al. [23]. The BC was made by pyrolysing grain husks and paper fibre sludge at 450–500 °C for 20 min; 60% of the particles had a size of below 2 mm (Sonnenerde Gmbh, Austria). In the <2 mm fraction the particle size distribution was <6.6 μm: 1.57%, 6.6–52.5 μm: 13.9%, 52.5–2000 μm: 84.52% [24]. The compost, which included green waste and sewage sludge from municipalities, was produced by FCC Hungary Inc., Gyál (Table 1).

Table 1. Physical and chemical properties of the acidic sandy soil, biochar (BC) and compost.

Parameter	Soil	BC	Compost
pH(H_2O)	4.9	10.4	7.08
OM%	0.64	22.5 *	8.12
$CaCO_3$%	0	5.75	6.81
Total N%	0.044	0.959	1.16
Total P (mg/kg)	260	6742	10,259
Total K (mg/kg)	1193	15,380	8243
Plant-available K (AL-K_2O, mg/kg)	36.1	12,595	4778
Plant-available P (AL-P_2O_5, mg/kg)	68.9	5227	8196
Total Ca (mg/kg)	309	34,270	54,207
Total Mg (mg/kg)	1096	3539	9161
Total Zn (mg/kg)	41.6	53.3	449

* Measured by the Walkley-Black method. The total carbon content, determined by incinerating the BC, was 60.4%; AL—ammonium-lactate soluble.

The bacterial inoculum (non-commercial product from Biofil Ltd. Hungary, based on patent No. WO 2015/118516) [25] consisted of PGPR isolated from sandy soils in Hungary. According to Patent WO 2012/093374, the composition of the conventional inoculant carrier (IC) is a mixture of perlite, zeolite and diatomite (1:0.6:0.9 ratio) with Vivapur® 101 microcrystalline cellulose as additive [26]. The PGPRs were mixed in the following ratio: *Bacillus aryabhattai*—LU44 (function: phosphate solubilisation; 3.4×10^8 CFU (colony-forming unit)/g IC); *Azospirillum brasilense*—NF7 (function: nitrogen fixation; 1.4×10^7 CFU/g IC); *Azospirillum brasilense*—242/9 (function: nitrogen fixation; 4.4×10^8 CFU/g IC); *Paenibacillus peoriae*—S284 (function: antimicrobial inhibition, nitrogen fixation; 2.4×10^7 CFU/g IC); *Arthrobacter crystallopoietes*—S153 (function: siderophore activity; 1×10^9 CFU/g IC). The PGPR combination was selected to intensify humification and soil aggregate formation, to improve the nitrogen, phosphorus and iron supply and to provide plant growth-promoting compounds under sandy and/or acidic soil conditions [19]. The selected strains have wide pH tolerance, which is favourable in the presence of biochar, since the latter tends to generate alkaline conditions.

2.2. Experiment Setup

Two experimental layouts were used to test the materials in soil: a completely randomised block for testing the separate effects of the additives, and a Box-Wilson method for testing their combined effects. The essence of this last method is that by changing the amendment doses in a specific order the number of treatment combinations can be significantly reduced [27]. In both experiments the pots contained 1.5 kg soil (pot volume: 1l). The highest dose of additives used in separate applications was determined on the basis of the quantities used in practice. Based on earlier experiments [28] the applied maximum dose was 45 t/ha = 1.5%wieght/weight (%*w*/*w*) for BC, while the manufacturer recommended a maximum dose of 15 t/ha = 0.5%*w*/*w* for compost. The doses of PGPR were determined by preliminary experiments performed on maize by the manufacturer. The lower doses were tested because measurable effects may already occur at these application rates on this low fertility soil. The treatment combinations in the experiments are shown in Table 2. The highest dose of BC and PGPR was the same in the separate and combined treatments, but the highest dose of compost in the combined treatments was only 0.33%*w*/*w*, to prevent the compost from suppressing the expected effects of the other amendments. Each treatment was performed in three or 10 replicates according to the experimental layout (Table 2). The soil and compost were air-dried, then sieved through a 2 mm mesh.

Table 2. Biochar (BC), compost and plant growth-promoting rhizobacteria (PGPR) treatments applied in the experiment. Separate applications: only one material added to the soil in different doses; combined application: at least two materials added to the soil in different doses. ("n": number of replications for each treatment level).

Treatment	Treatment Level Code	Amounts of Each Material Applied		
		BC (%*w*/*w*)	PGPR (CFU/pot)	Compost (%*w*/*w*)
SEPARATE APPLICATION (completely randomised block design)				
Control treatment (n = 3)	-	0	0	0
Compost treatments (n = 3)	C1	0	0	0.16
	C2	0	0	0.33
	C3	0	0	0.5
BC treatments (n = 3)	BC1	0.5	0	0
	BC2	1	0	0
	BC3	1.5	0	0
PGPR treatments (n = 3)	PGPR1	0	3.7×10^6	0
	PGPR2	0	7.5×10^6	0
	PGPR3	0	1.2×10^7	0
COMBINED APPLICATION (Box and Wilson design)				
Alternating treatments (n = 3)	1	1.5	1.2×10^7	0.25
	2	0.5	1.2×10^7	0.25
	3	1.5	3.7×10^6	0.25
	4	0.5	3.7×10^6	0.25
	5	1.5	1.2×10^7	0.08
	6	0.5	1.2×10^7	0.08
	7	1.5	3.7×10^6	0.08
	8	0.5	3.7×10^6	0.08
Extreme treatments (n = 3)	9	2	7.5×10^6	0.16
	10	0	7.5×10^6	0.16
	11	1	2.4×10^7	0.16
	12	1	0	0.16
	13	1	7.5×10^6	0.33
	14	1	7.5×10^6	0
Central treatment (n = 10)	15	1	7.5×10^6	0.16

The treatments were set up in 11 pots, 13 cm in height, 13 cm wide at the top and 9 cm at the base. The bottoms of the pots were sealed so that no leaching occurred during the experiment. The component(s) of a given treatment were mixed thoroughly with 1.5 kg soil, then placed in the pots and wetted to 65% of maximum field capacity. Each pot was then weighed and kept in a dark room for two weeks at a temperature of 20 °C for incubation. During incubation and plant growth the water loss was monitored by the gravimetric method twice a week and the missing moisture was replaced by irrigation. According to Kang et al. [29] 65% of field capacity can be considered as a satisfactory moisture content for maize growth. After incubation, the pots were placed in a growth chamber with a 12/12 h photoperiod and a temperature setting of 26/16 °C, representing day (600 μmol/m^2/s photon flux density) and night phases. The test plant was maize (*Zea mays* L., Mv 277), for which the tested soil was relatively unfavourable, so more pronounced treatment effects could be expected [30]. Two dressed seeds of maize were sown in each pot, the less developed of which was removed after germination. At the end of the two-month growth period the above-ground biomass of the plants was harvested and weighed, after which soil and plant samples were prepared for analysis.

2.3. Chemical and Biological Analysis

The pH was measured according to ISO 10390:2005 in a 1:2.5 soil:water suspension 12 h after mixing [31]. The OM content was determined using the modified Walkley-Black method [32]. The organic carbon content of BC was measured by incineration [33]. The $CaCO_3$ content was measured using the Scheibler gas-volumetric method [34]. The carbonates present in the sample were converted into CO_2 by the addition of hydrochloric acid. The plant-available phosphorus (P) and potassium (K) contents in the soil were determined in ammonium-acetate lactate extract (AL-P_2O_5, AL-K_2O) using the Egner–Riehm–Domingo method [35]. The total nitrogen (N) content of soil and plants was determined with the Kjeldahl method [36], digesting the organic matter so that both total organic and inorganic N content could be measured. The NH_4-N and NO_3-N contents were measured in KCl extracts using the titrimetric method [35]. The pseudo total element contents were determined with the ICP-AES method (Jobin-Yvon Ultima 2) after microwave teflon bomb digestion with aqua regia [37] using Merck calibration standards and following the manufacturer's instructions. In each ICP measurement session the extract of a standard soil sample was also analysed as a control. The calibration curves were determined after every 12th sample.

Substrate-induced respiration (SIR) [38] was measured according to Szili-Kovács et al. [39]. Samples with a 2:1 water to soil ratio were incubated after the addition of glucose. The intensity of AMF colonization (M%) and the arbusculum richness (A%) in the roots were calculated using a five-class system [40] after observing 30 randomly selected root segments, each 1 cm in length. Root samples from the pots were cleared in KOH solution (15%*w*/*w*) and stained with aniline blue [41].

2.4. Statistical Analysis

The separate treatment effects were analysed using one-way ANOVA. Significant differences between the treatment means were calculated using the least significant difference (LSD) test at the $p < 0.01$, $p < 0.05$ and $p < 0.1$ levels. The results of the combined applications were evaluated using analysis of variance and regression analysis [28]. As the coefficient of determination (R^2) shows whether the model fits the data, only variables for which the Box-Wilson model gave R^2 values higher than 60% are discussed here, since this indicates that changes in these variables could be explained to at least a moderate extent by the model equation. The variance of these variables was determined using the F-test. Variability between the samples was determined by means of principal component analysis (PCA). Statistica v.9 (StatSoft Inc., Tulsa, OK, USA) software was used for the statistical evaluation.

3. Results

3.1. Soil pH and OM Content

The application of 1.5%*w*/*w* BC alone significantly increased the soil pH to 5.9, while it rose to 5.5 in the 0.5%*w*/*w* compost treatment (Tables 3–5). Table 6 presents the R^2 and p values for parameters exhibiting R^2 values greater than 60% in the combined treatments, while Table 7 contains the mean values and $LSD_{5\%}$ values of these parameters for each treatment combination. In combined treatments compost and PGPR had an additive effect, but BC was decisive for pH change due to its application rate (Tables 6 and 7).

Table 3. Significant effect of individual biochar (BC) treatment levels (%*w*/*w*) on the properties of acidic sandy soil and maize. Data are mean ± sd of the replicates.

Properties	Levels				$LSD_{5\%}$	*p*
	Control	BC1	BC2	BC3		
Soil						
pH (H_2O)	4.93 ± 0.1	5.29 ± 0.02	5.71 ± 0.07	5.94 ± 0.05	0.18	***
OM%	0.642 ± 0.06	0.67 ± 0.06	0.70 ± 0.06	0.77 ± 0.05	0.10	*
Total K mg/kg	1193 ± 14	1261 ± 217	1386 ± 85	1521 ± 171	148	***
AL-K_2O mg/kg	36.1 ± 4.0	62.8 ± 1.1	93.0 ± 4.3	145.3 ± 8.9	10.2	***
Total P mg/kg	260 ± 2	279 ± 14	301 ± 19	302 ± 12	36	*
AL-P_2O_5 mg/kg	68.9 ± 1.3	85.9 ± 7.8	101.2 ± 7.5	111.3 ± 8.6	13.8	***
NO_3-N mg/kg	1.93 ± 0.20	2.27 ± 0.00	3.29 ± 0.17	3.75 ± 0.24	0.50	***
SIR (μg CO_2-C/g soil/hour)	0.72 ± 0.07	0.94 ± 0.12	1.07 ± 0.06	1.12 ± 0.10	0.25	**
Maize						
AMF–M%	52.1 ± 2.3	59.3 ± 2.0	58.4 ± 8.2	76.1 ± 6.8	9.5	**
AMF–A%	38.6 ± 1.5	49.0 ± 5.0	48.2 ± 5.0	64.1 ± 5.4	9.9	**
Maize P mg/kg	1596 ± 233	2196 ± 98	3096 ± 144	3865 ± 358	625	***
Maize K mg/kg	10,804 ± 1184	24,270 ± 1050	32,728 ± 305	37,034 ± 1387	2913	***

BC levels: BC1: 0.5 *w*/*w*%; BC2: 1 *w*/*w*%; BC3: 1.5 *w*/*w*%; AL—ammonium-lactate soluble; SIR: substrate-induced respiration; AMF-M and AMF-A: arbuscular mycorrhizal fungi, intensity of colonization and arbusculum richness; $LSD_{5\%}$: least significant difference at $p < 0.01$: ***; $p < 0.05$: **; $p < 0.1$: *.

Table 4. Significant effect of individual compost treatment levels (%*w*/*w*) on the properties of acidic sandy soil and maize. Data are mean ± sd of the replicates.

Properties	Levels				$LSD_{5\%}$	*p*
	Control	C1	C2	C3		
Soil						
pH (H_2O)	4.93 ± 0.1	5.08 ± 0.03	5.32 ± 0.01	5.51 ± 0.03	0.14	***
Total K mg/kg	1193 ± 14	1255 ± 136	1144 ± 73	1353 ± 108	139	**
Total P mg/kg	260 ± 2	273 ± 5	286 ± 4	294 ± 6	12	***
AL-P_2O_5 mg/kg	68.9 ± 1.3	78.8 ± 2.1	101.3 ± 4.7	120.7 ± 7.0	10.5	***
Total N %*w*/*w*	0.044 ± 0.002	0.039 ± 0.002	0.040 ± 0.002	0.041 ± 0.000	0.003	**
NO_3-N mg/kg	1.93 ± 0.20	0.73 ± 0.21	0.87 ± 0.36	0.90 ± 0.37	0.71	**
SIR (μg CO_2-C/g soil/hour)	0.72 ± 0.07	1.06 ± 0.11	1.12 ± 0.12	1.40 ± 0.10	0.28	***
Maize						
AMF–A%	38.6 ± 1.5	24.5 ± 4.4	17.5 ± 1.4	9.3 ± 1.8	5.7	***
Maize dry biomass (g/pot)	2.68 ± 0.29	4.53 ± 0.21	5.89 ± 0.48	7.30 ± 0.25	0.75	***
Maize N %*w*/*w*	0.478 ± 0.013	0.379 ± 0.011	0.389 ± 0.034	0.421 ± 0.015	0.052	**

Compost levels: C1: 0.16 *w*/*w*%; C2: 0.33 *w*/*w*%; C3: 0.5 *w*/*w*%; AL—ammonium-lactate soluble; SIR: substrate-induced respiration; AMF-A: arbusculum richness of arbuscular mycorrhizal fungi; $LSD_{5\%}$: least significant difference at $p < 0.01$: ***; $p < 0.05$: **.

Table 5. Significant effect of individual plant growth-promoting rhizobacteria (PGPR) treatment levels (colony forming units (CFU)/pot) on the properties of acidic sandy soil and maize. Data are mean ± sd of the replicates.

Properties	Levels				$LSD_{5\%}$	*p*
	Control	PGPR1	PGPR2	PGPR3		
Soil						
AL-K_2O mg/kg	36.1 ± 4.0	27.5 ± 1.8	30.7 ± 0.9	24.4 ± 1.1	6.3	**
Maize						
AMF–M%	52.1 ± 2.3	64.8 ± 0.5	65.7 ± 6.3	71.6 ± 4.4	10.4	*
Maize P mg/kg	1596 ± 233	1861 ± 66	2020 ± 76	2033 ± 249	362	*
Maize K mg/kg	10,804 ± 1184	12,580 ± 102	14,007 ± 373	13,414 ± 523	1302	***

PGPR levels: PGPR1: 3.7 × 10^6 CFU/pot; PGPR2: 7.5 × 10^6 CFU/pot; PGPR3: 1.2 × 10^7 CFU/pot; AL—ammonium-lactate soluble; AMF-M: arbuscular mycorrhizal fungi, intensity of colonization; $LSD_{5\%}$: least significant difference at $p < 0.01$: ***; $p < 0.05$: **; $p < 0.1$: *.

The OM% of BC, measured with the Walkley-Black method, was almost three times higher than that of compost and can be considered as a labile fraction that can be mineralised in the soil [42]. The maximum dose of BC alone caused a 19% increase in OM%. There was no significant OM% increase in response to compost alone, because the standard deviation of the OM% values (0.06%) exceeded the OM increase that could be expected in compost treatments (0.01–0.04%) (Table 3). The measured OM% increment in treatments with BC alone were 70% lower on average than the expected value based on the amount of OM added (Figure 1). This means that, on average, the soil OM content in BC-treated soils after harvest was 21% lower than expected. In the combined treatments (1–9, 11, 13 and 15) when compost and PGPR were applied with BC, around 100% of the added OM could be found in the soil after harvesting the maize biomass. The exception was treatment No. 9, in which the highest BC dose (2%) was applied (Figure 1). In the case of treatments 4 and 7 the high OM values measured may have been caused by undetectable plant residues in the soil sample.

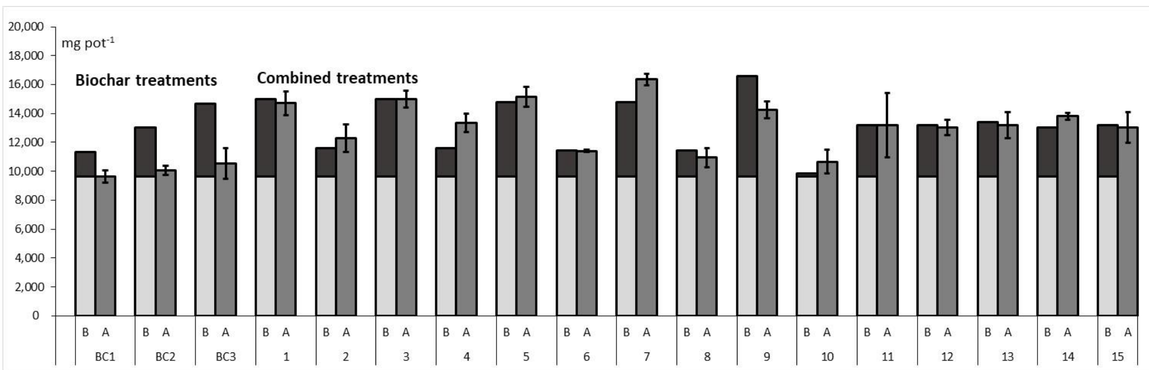

Figure 1. Comparison of soil OM content measured at the beginning of (B) and after (A) the experiment in the different treatments. Treatments 1–15: combined treatments, BC1–3: separate biochar treatments. Treatment doses can be seen in Table 2. Legend: light grey: original soil OM content; dark grey: OM added in individual treatments; medium grey: OM content measured in the soil after the experiment.

Table 6. Coefficient of determination (R^2) and p values for parameters with R^2 values greater than 60% in the combined treatments.

Treatment	Soil								Maize	
	pH (H_2O)	OM (%)	AL-K_2O (mg/kg)	Total P (mg/kg)	AL-P_2O_5 (mg/kg)	NO_3-N (mg/kg)	Ratio of Plant-Available K to Total K (%)	Ratio of Plant-Available P to Total P (%)	K (mg/kg)	P (mg/kg)
BC	+ ***	+ ***	+ ***	+ ***	+ ***	+ ***	+ ***	+ ***	+ ***	+ ***
PGPR										
Compost				+ *	+ **			+ ***		
BC × PGPR									+ *	
BC × Compost		+ **								
Compost × PGPR										
R^2	92.56	62.86	93.59	70.46	83.79	63.98	89.47	75.71	81.77	74.11

BC—biochar; PGPR—plant growth-promoting rhizobacteria; +—positive effect; $p < 0.01$: ***; $p < 0.05$: **; $p < 0.1$: * AL—ammonium-lactate soluble.

Table 7. Mean values of significant parameters and $LSD_{5\%}$ values from the biochar (BC), compost and plant growth-promoting rhizobacteria (PGPR) combined treatments. Data are mean ± sd of the replicates.

Treatment	Soil								Maize	
	pH (H_2O)	OM (%)	AL-K_2O (mg/kg)	Total P (mg/kg)	AL-P_2O_5 (mg/kg)	NO_3-N (mg/kg)	Ratio of Available K to Total K Content (%)	Ratio of Available P to Total P Content (%)	K (mg/kg)	P (mg/kg)
1	6.4 ± 0.1	0.98 ± 0.04	196.7 ± 6.9	342 ± 9	184 ± 14.2	4.75 ± 0.49	11.14 ± 0.67	23.7 ± 1.58	61,566 ± 4210	3300 ± 500
2	5.7 ± 0.0	0.82 ± 0.05	93.2 ± 4.0	307 ± 9	122.0 ± 2.2	2.36 ± 0.21	6.09 ± 0.14	17.5 ± 0.40	38,474 ± 360	1627 ± 135
3	6.3 ± 0.0	1.00 ± 0.03	184.7 ± 4.5	356 ± 11	188.0 ± 5.0	3.79 ± 0.25	10.69 ± 0.29	23.2 ± 1.37	51,718 ± 307	2804 ± 63
4	5.7 ± 0.0	0.89 ± 0.03	81.7 ± 7.6	308 ± 9	129.7 ± 7.7	2.23 ± 0.39	5.93 ± 0.67	18.5 ± 1.09	39,365 ± 4768	2046 ± 290
5	6.2 ± 0.0	1.01 ± 0.04	177.3 ± 8.1	352 ± 4	166 ± 13.3	4.41 ± 0.36	11.13 ± 0.38	20.8 ± 1.47	47,364 ± 1062	3352 ± 144
6	5.4 ± 0.0	0.76 ± 0.00	74.9 ± 5.8	286 ± 7	102.0 ± 2.4	2.38 ± 0.54	5.94 ± 0.56	15.7 ± 0.44	36,828 ± 1842	2101 ± 274
7	6.2 ± 0.0	1.09 ± 0.02	153.7 ± 8.7	320 ± 8	147.3 ± 2.4	4.01 ± 0.41	9.75 ± 0.73	20.3 ± 0.73	46,200 ± 3020	3216 ± 334
8	5.4 ± 0.0	0.73 ± 0.04	67.5 ± 3.8	280 ± 6	101.5 ± 2.7	3.49 ± 0.25	4.61 ± 0.70	16.0 ± 0.76	38,245 ± 2988	2305 ± 88
9	6.3 ± 0.1	0.95 ± 0.03	199 ± 13.7	344 ± 13	169 ± 15.3	5.44 ± 0.12	13.56 ± 1.71	21.7 ± 1.15	48,114 ± 3068	3612 ± 425
10	5.1 ± 0.1	0.71 ± 0.04	49.7 ± 3.4	269 ± 8	93.2 ± 1.3	1.76 ± 0.38	3.64 ± 0.32	15.2 ± 0.57	22,846 ± 566	1422 ± 273
11	6.1 ± 0.2	0.88 ± 0.12	130 ± 10.0	311 ± 9	127 ± 13.5	5.18 ± 0.40	8.16 ± 0.51	18.1 ± 1.71	47,896 ± 2853	2740 ± 228
12	5.9 ± 0.1	0.87 ± 0.03	128.7 ± 9.7	304 ± 9	134.0 ± 5.1	6.03 ± 0.75	8.05 ± 0.88	19.4 ± 0.83	49,954 ± 1638	2645 ± 460
13	6.1 ± 0.1	0.88 ± 0.05	128.7 ± 5.7	313 ± 5	162 ± 16.9	4.95 ± 0.42	7.83 ± 0.29	22.8 ± 2.62	45,830 ± 2825	2275 ± 114
14	5.9 ± 0.1	0.92 ± 0.01	119 ± 10.2	293 ± 7	114.0 ± 4.2	4.45 ± 0.60	7.80 ± 0.64	17.1 ± 1.03	48,398 ± 1562	2721 ± 620
15	6.0 ± 0.1	0.87 ± 0.07	115.6 ± 8.5	312 ± 18	132.2 ± 6.7	3.80 ± 0.72	7.73 ± 0.90	18.6 ± 0.98	44,006 ± 3180	2724 ± 291
$LSD_{5\%}$	0.1	0.08	11.3	17	12.5	0.74	0.54	0.82	3933	449

AL—ammonium-lactate soluble; $LSD_{5\%}$: least significant difference at $p < 0.05$.

3.2. N, P and K Contents in Soil and Plants

BC2 and BC3 treatments significantly increased the plant-available NO_3-N and the total and plant-available P and K contents in the soil, but not that of total N (Table 3). PGPR application caused a decrease in plant-available K in the soil, while compost increased the total P, K and plant-available P contents. In response to BC alone the AL-P_2O_5 and AL-K_2O contents rose to a greater extent than the total P and K contents in soil, since about 80% of both the P and K content was in plant-available form (Tables 1 and 3). In these treatments the P and K contents also increased in maize (Tables 3–5). Although compost raised the K (treatment C3) and P (treatments C1–3) contents of the soil, there was no significant change in the plant P and K contents. However, the K and P uptake of maize increased almost three and two times, respectively, in the 0.5%*w*/*w* compost treatment compared to the control (data not shown). The inoculum itself was capable of improving the nutrient-supplying ability of the soil: as a result of PGPR treatment the plant P and K concentrations rose significantly (Table 5).

The combined treatments indicated that BC was decisive for changes in the ratio of plant-available K to total K content, as it increased plant-available K to a greater extent (Tables 6 and 7). Both BC and compost led to a rise in the ratio of plant-available P to total P content, indicating increased P availability (Table 6).

Compost and PGPR had different effects on P and K availability when applied alone or in combined treatments. Compost alone did not affect the ratio of available and total K in the soil compared to the control (ratio: 2.4%), while the inoculum decreased it to a value of 1.6%. However, when combined with 1.5%*w*/*w* BC (combined treatments 1, 3, 5 and 7) both compost and PGPR improved P and K availability. In these treatments the ratio of available to total K was 35% higher on average (10.7%) compared to 1.5%*w*/*w* BC alone (7.9%). A 35% difference was also found in these treatments for P availability.

In the combined treatments the P and K contents of the maize biomass were primarily dependent on the BC treatment, but the K content was also influenced by PGPR (Table 6).

Regarding soil N, there was no significant change in the NH_4-N content (14 mg/kg in the control soil), but the NO_3-N content rose significantly in response to BC2 and BC3 treatments and decreased significantly after treatment with compost alone (treatments C1–3) (Tables 3 and 4). The combined treatments revealed that the change in soil NO_3-N content was mainly influenced by BC (Table 6). The C1–3 treatments decreased the maize N content, but the N uptake tripled in the 0.5%*w*/*w* (treatment C3) treatment compared to the control.

3.3. Maize Biomass, SIR and AMF Colonisation

In the separate treatments only compost (treatments C1–3) significantly increased the maize biomass. In the combined treatments there was no significant difference between the dry weights of the plants (Tables S1 and S2). The average biomass in the combined treatments was 2.9 g dry matter/pot, which was statistically equal to the value of the control treatment (Table 3).

The 1.5%*w*/*w* dose of BC alone resulted in a 1.5 times increase in SIR, while the 0.5%*w*/*w* dose of compost alone doubled it (Tables 3 and 4). SIR was not influenced by PGPR addition. The combined application of the materials led to an increase in microbial biomass compared to the untreated soil (data not shown due to the low coefficient of determination). The lowest SIR value (1.18 ± 0.03 μg CO_2-C/g soil/hour) was recorded in treatment 2, given a 0.5%*w*/*w* BC dose combined with 0.25%*w*/*w* compost and PGPR, and the highest (1.86 ± 0.22 μg CO_2-C/g soil/hour) in treatment 7, given a 1.5%*w*/*w* BC dose with 0.08%*w*/*w* compost and PGPR (Table 2).

The application of BC alone caused an increase in the colonization intensity in treatment BC3 (M%) and arbuscular richness in treatments BC1–3 (A%) of indigenous AMF, while the arbuscular richness declined considerably in soils treated with compost (treatments C1–3) (Tables 3 and 4). In combined applications the mycorrhizal parameters indicated that the infectivity of the indigenous AMF community had been inhibited (data not shown due to the low coefficient of determination).

Even the highest value of M% (17.84) was considerably lower than in any of the treatments where BC, compost and PGPR were applied alone.

4. Discussion

Each of the materials applied had several positive effects on soil properties, but only compost resulted in an improvement in soil fertility, defined here as the plant biomass produced on it. Differences in early growth stages may determine the final biomass and yield of maize [43]. Thus, in contrast with expectations, the combined application of BC, compost and PGPR had no positive synergistic effect on soil fertility on this acidic sandy soil in the short term, when the water supply was satisfactory for maize [4,21,22,30]. The failure of BC to influence soil fertility can probably be attributed to the short experiment time and the laboratory conditions [44].

Though BC is basically used to amend physical soil properties, in the short term it may significantly influence chemical soil properties, though this depends on the type of BC [4,12,22]. In a similar short-term pot experiment, reported by Wang et al. [14], the application of BC without fertiliser resulted in plant yield depression. In the present experiment this adverse effect was not observed for BC alone, probably due to its significant labile fraction, which provided nutrients, thus avoiding the need for fertiliser addition.

BC and compost influenced the availability of nutrients in three ways: through their own nutrient and OM content, their pH-enhancing effect and the changes induced in the activity of the microbes present in the soil. PGPR exerted its effect by influencing the soil microbial community.

The most important of the possible longer term effects of the amendments is their effect on soil OM. Although BC is a recalcitrant material, it may induce the decomposition of soil OM due to its labile fraction. The application of BC alone probably triggered a positive priming effect in the soil, causing the native OM to be mineralised and resulting in lower OM% than in combined treatments with compost and PGPR. The 21% discrepancy between measured OM% and the expected value fits into the range described by Whitman et al. [45]. The OM% of the BC was relatively high (22.5%), and this labile fraction can be mineralised in the soil within a short time [46,47]. The N content of the BC was also comparatively high (0.96%), which may also have facilitated positive priming in the soil [6,48]. In the combined treatments the priming could have been inhibited by PGPR, which had an antibacterial impact due to the presence of *Paenibacillus peoriae*, and may thus have had a negative effect on the soil microbe community [49]. Compost may also have alleviated priming by decreasing the C/N ratio of the added organic materials in the soil [50].

BC and compost had similar $CaCO_3$% contents, so both materials increased the soil pH due to the liming effect. This may be the main beneficial effect on soil fertility on this acidic sandy soil, as also reported in the study of van Zwieten et al. [11]. As a result, the liming effect directly influenced the N cycle in the soil. Both BC and compost influenced the NO_3-N content, but in contrasting ways as a function of their liming effect. Ammonification is a less pH-dependent process than nitrification, so the higher pH in the BC treatments could have facilitated an increase in NO_3-N content, while NH_4-N remained unchanged [19]. In the case of compost the slighter pH increment could have resulted in less intensive nitrification. In response to compost the NO_3-N content in the soil declined, which could be attributed to plant uptake, since a considerable quantity of plant biomass was formed in this treatment, resulting in greater N uptake [51]. The ability of BC to promote nitrification and raise pH was also manifested in the combined treatments, resulting in higher NO_3-N contents than when BC was applied alone, due to the joint N content of compost and BC [52]. In the combined treatments the high C/N ratio of BC could have led to the immobilisation of N, resulting in limited uptake by maize [52]. Despite the satisfactory P and K content, this may have inhibited biomass growth.

The increase in soil pH also had a certain effect on P and K availability. In combined treatments changes in the P and K concentrations in plant biomass and soil were mostly related to the nutrient content of BC, the pH-enhancing effect of which resulted in the optimum pH range for nutrient availability and plant uptake [44,53]. These treatments not only supplied rapidly utilisable carbon sources from the compost, but also provided protection to microorganisms due to the pore volume of a high dose of BC [54]. The consequent higher microbial activity and mineralisation could explain the 35% higher P and K availability values in these treatments compared to BC alone.

The exogenous OM provided by compost was more favourable for microbial decomposition, having a lower C/N ratio than BC. In addition, the compost itself contained a substantial number of microorganisms, resulting in higher SIR values in compost treatments than in BC treatments [5,55]. As also reported by Hussain [4], the microbial activity was higher in treatments with BC, compost and PGPR than when these materials were applied alone, but no significant differences were observed between the combined treatments. There was also a significant increase in plant biomass in the compost treatment, probably associated with the greater quantity of roots and root exudates, which may also have increased the microbial biomass in this treatment [56].

The ability of compost to influence P content and availability could be related to its high P content, but could also be attributed to the fact that the enhanced microbial activity induced by the compost solubilised organically bound P [57]. Separate PGPR application was also able to mobilise the soil P content through the activity of mineral phosphate-solubilising strains (*Bacillus aryabhattai*) [58], though this effect was only manifested in the maize P content. Although the inoculum did not contain K-solubilising bacteria, it promoted K uptake by maize and might have created more favourable conditions for mycorrhizal symbiosis (Table 5), thus enhancing both P and K mobilisation and plant uptake. In combined treatments this strengthened the effect of BC on the AL-K_2O content (Table 6) [59,60], but for the other soil properties the effect of inoculum was suppressed by that of compost and BC, as also observed by Ohsowski et al. [22].

The availability of P has a direct effect on the AMF colonization of the roots. The compost treatment significantly decreased the value of AMF-A%, indicating that an improvement in nutrient supplies could substantially reduce the dependence of plants on symbiotic organisms [61]. BC may stimulate symbiosis on such low fertility soil, but high soil P content may inhibit AMF infectivity and colonisation [62]. When BC was applied alone symbiosis was stimulated, but in combined treatments the enhanced nutrient availability limited fungal growth.

PCA was performed on all the treatments to obtain a better understanding of the interactions taking place in the combined treatments, and the data showed that two principal factors explained 68.61% of the variance (Figure 2).

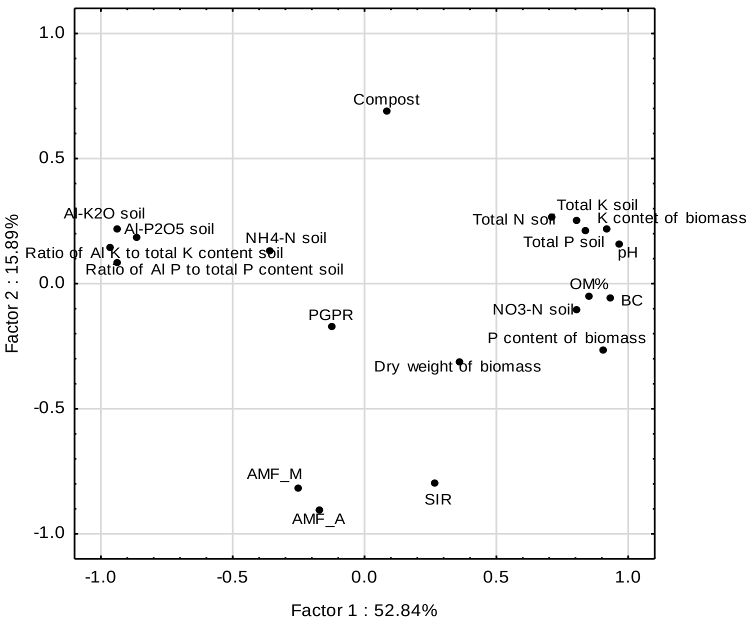

Figure 2. Biplot of the first two principal components of the soil and maize properties and biochar (BC), Compost and plant growth-promoting rhizobacteria (PGPR) treatment.

While Factor1 correlated primarily with BC, Factor2 correlated with compost treatment. Except in the case of AL-K_2O, significant changes that could be attributed to PGPR were only observed when it was applied alone. These results show that BC had a significant effect on macronutrient availability, which is decisive for plant production. The compost had the greatest influence on the soil biota, which may be related to the easier mineralisation of the organic compounds in this material due to its lower C/N ratio [16].

5. Conclusions

The main benefit of the combined application of BC, compost and PGPR on this acidic sandy soil was that it prevented the positive priming effect observed when BC was applied alone. When combined with BC, compost and PGPR increased the ratio of available to total P and K concentrations via the intensification of soil microbial activity, while compost and PGPR alone did not increase P and K availability. The microbial activity in the soil was mainly stimulated by the OM content of BC and compost and by the pH changes they caused, while the negative influence of the high P content of compost on the AMF parameters was mitigated by BC. In a sandy soil of this type, if the water supplies are adequate, an increase in biomass can only be achieved in the short term in response to the easily accessible nutrient content of the compost, while BC and PGPR are ineffective in this respect at the applied doses. When applied in combination with BC, the ability of compost to increase plant biomass may be counteracted by N immobilisation by BC, so on BC-amended soils with adequate water supplies it may be necessary to use more than the recommended doses of compost or other organic fertilisers in order to increase yields. Further research in connection with the use of biochar on low fertility soil will need to include a wider range of organic fertiliser and inoculum doses. Experiments carried out under field conditions over a number of growing periods can be expected to give a better picture of the possible synergistic effects of the tested materials on soil fertility, which could not be detected in the present study.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/10/1612/s1, Table S1: Dry biomass weight from the separate application experiment, Table S2: Dry biomass weight from the combined application experiment.

Author Contributions: Conceptualization, N.U., J.K., M.M. and M.R.; methodology, N.U., J.K. and M.R.; software, L.R.; validation, N.U., J.K. and M.R.; formal analysis, E.D.; investigation, L.R., A.F., T.S.-K., E.D. and É.F.; resources, N.U.; data curation, N.U.; writing—original draft preparation, N.U., A.F., T.T., T.S.-K. and M.R.; writing—review and editing, J.K. and N.S.-V.; visualization, A.F.; supervision, N.U.; project administration, N.U.; funding acquisition, N.U., M.M. and J.K.; All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Norway Grant HU09-0029-A1-2013 entitled Combined application of biochar and microbial inoculant for deteriorated soils.

Conflicts of Interest: The authors declare that there is no conflict of interest.

References

1. Aranyos, J.T.; Tomócsik, A.; Makádi, M.; Mészáros, J.; Blaskó, L. Changes in physical properties of sandy soil after long-term compost treatment. *Int. Agrophys.* **2016**, *30*, 269–274. [CrossRef]
2. Pare, T.; Gregorich, E.G. Soil textural effects on mineralization of nitrogen from crop residues and the added nitrogen interaction. *Commun. Soil Sci. Plant Anal.* **1999**, *30*, 145–157. [CrossRef]
3. Tahir, S.; Marschner, P. Clay addition to sandy soil reduces nutrient leaching—Effect of clay concentration and ped size. *Commun. Soil Sci. Plant Anal.* **2017**, *48*, 1813–1821. [CrossRef]
4. Hussain, F.; Hussain, I.; Khan, A.H.A.; Muhammad, Y.S.; Iqbal, M.; Soja, G.; Reichenauer, T.G.; Zeshan; Yousaf, S. Combined application of biochar, compost, and bacterial consortia with Italian ryegrass enhanced phytoremediation of petroleum hydrocarbon contaminated soil. *Environ. Exp. Bot.* **2018**, *153*, 80–88. [CrossRef]
5. Sadet-Bourgeteau, S.; Hout, S.; Karimi, B.; Mathieu, O.; Mercier, V.; Montenach, D.; Morvan, T.; Sappin-Didier, V.; Watteau, V.; Nowak, V.; et al. Microbial communities from different soil types respond differently to organic waste input. *Appl. Soil Ecol.* **2019**, *143*, 70–79. [CrossRef]

6. Verheijen, F.; Jeffery, S.; Bastos, A.C.; Van Der Velde, M.; Diafas, I. *Biochar Application to Soils—A Critical Scientific Review of Effects on Soil Properties, Processes and Functions*; EUR 24099 EN; Office for the Official Publications of the European Communities: Luxembourg, 2010; p. 149, ISBN 978-92-79-14293-2. [CrossRef]
7. Thies, J.E.; Rillig, M.C. *Characteristics of Biochar: Biological Properties, In Biochar for Environmental Management: Science and Technology*; Lehmann, J., Joseph, S., Eds.; Earthscan Books Ltd.: London, UK; New York, NY, USA, 2009; pp. 85–105, ISBN 978-1-84407-658-1.
8. Egamberdieva, D.; Hua, M.; Reckling, M.; Wirth, S.; Bellingrath-Kimura, S.D. Potential effects of biochar-based microbial inoculants in agriculture. *Environ. Sustain.* **2018**, *1*, 19–24. [CrossRef]
9. Kuzyakov, Y.; Subbotina, I.; Chen, H.; Bogomolova, I.; Xu, X. Black carbon decomposition and incorporation into soil microbial biomass estimated by 14C labeling. *Soil Biol. Biochem.* **2009**, *41*, 210–219. [CrossRef]
10. Zimmerman, A.R.; Gao, B.; Ahn, M.-Y. Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biol. Biochem.* **2011**, *43*, 1169–1179. [CrossRef]
11. Van Zwieten, L.; Kimber, S.; Morris, S.; Chan, K.Y.; Downie, A.; Rust, J.; Joseph, S.D.; Cowie, A. Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant Soil* **2010**, *327*, 235–246. [CrossRef]
12. El-Naggar, A.; Lee, S.S.; Rinklebe, J.; Farooq, M.; Song, H.; Sarmah, A.K.; Zimmerman, A.R.; Ahmad, M.; Shaheen, S.M.; Ok, Y.S. Biochar application to low fertility soils: A review of current status, and future prospects. *Geoderma* **2019**, *337*, 536–554. [CrossRef]
13. Bista, P.; Ghimire, R.; Machado, S.; Pritchett, L. Biochar effects on soil properties and wheat biomass vary with fertility management. *Agronomy* **2019**, *9*, 623. [CrossRef]
14. Wang, Y.; Zhang, L.; Yang, H.; Yan, G.; Xu, Z.; Chen, C.; Zhang, D. Biochar nutrient availability rather than its water holding capacity governs the growth of both C3 and C4 plants. *J. Soils Sedim.* **2016**, *16*, 801–810. [CrossRef]
15. Cortellini, L.; Toderi, G.; Baldoni, G.; Nassisi, A. Effects on the content of organic matter, nitrogen, phosphorus and heavy metals in soil and plants after application of compost and sewage sludge. In *The Science of Composting*; de Bertoldi, M., Sequi, P., Lemmes, B., Papi, T., Eds.; Springer: Dordrecht, The Netherlands, 1996; pp. 457–468. [CrossRef]
16. Wu, H.; Zeng, G.; Liang, J.; Chen, J.; Xu, J.; Dai, J.; Li, V.; Chen, M.; Xu, P.; Zhou, Y.; et al. Responses of bacterial community and functional marker genes of nitrogen cycling to biochar, compost and combined amendments in soil. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 8583–8591. [CrossRef] [PubMed]
17. Brodowski, S.; John, B.; Flessa, H.; Amelung, W. Aggregate-occluded black carbon in soil. *Eur. J. Soil Sci.* **2006**, *57*, 539–546. [CrossRef]
18. Yadav, A. Microbial inoculants for sustainable agriculture. *Int. J. Curr. Microbiol. Appl. Sci.* **2018**, *7*, 800–804. [CrossRef]
19. Rékási, M.; Szili-Kovács, T.; Takács, T.; Bernhardt, B.; Puspán, I.; Kovács, R.; Kutasi, J.; Draskovits, E.; Molnár, S.; Molnár, M.; et al. Improving the fertility of sandy soils in the temperate region by combined biochar and microbial inoculant treatments. *Arch. Agron. Soil Sci.* **2018**, *65*, 44–57. [CrossRef]
20. Moland, S.; Robicheau, B.M.; Browne, R.; Newell, R.; Walker, A.K. Determining the effects of biochar and an arbuscular mycorrhizal inoculant on the growth of fowl mannagrass (*Glyceria striata*) (Poaceae). *FACETS* **2018**, *3*, 441–454. [CrossRef]
21. Nadeem, S.M.; Imran, M.; Naveed, M.; Khan, M.Y.; Ahmad, M.; Zahir, Z.A.; Crowley, D.E. Synergistic use of biochar, compost and plant growth-promoting rhizobacteria for enhancing cucumber growth under water deficit conditions. *J. Sci. Food Agric.* **2017**, *97*, 5139–5145. [CrossRef]
22. Ohsowski, B.M.; Dunfield, K.; Klironomos, J.N.; Hart, M.M. Plant response to biochar, compost, and mycorrhizal fungal amendments in post-mine sandpits. *Restor. Ecol.* **2018**, *26*, 63–72. [CrossRef]
23. Molnár, M.; Vaszita, E.; Farkas, É.; Ujaczki, É.; Fekete-Kertész, I.; Tolner, M.; Klebercz, O.; Kirchkeszner, C.; Gruiz, K.; Uzinger, N.; et al. Acidic sandy soil improvement with biochar—A microcosm study. *Sci. Total Environ.* **2016**, *563–564*, 855–865. [CrossRef]
24. Horel, A.; Barna, G.; Mako, A. Soil physical properties affected by biochar addition at different plant phaenological phases. Part I. *Int. Agrophys.* **2019**, *33*, 255–262. [CrossRef]
25. Soil Bacteriae for Inoculating Stress Soils. WO2015/118516. Available online: https://patentscope.wipo.int/ (accessed on 26 February 2017).

26. Soil Bacteria Containing Fertilizer and Method for Its Preparation. WO 2012/093374. Available online: https://patentscope.wipo.int/ (accessed on 26 February 2017).
27. Box, G.E.P.; Wilson, K.B. On the experimental attainment of optimum condition. *J. R. Stat. Soc. Ser. B.* **1951**, *13*, 1–38. [CrossRef]
28. Major, J. *Guidelines on Practical Aspects of Biochar Application to Field Soil in Various Soil Management Systems*; International Biochar Initiative: Washington, DC, USA, 2010. Available online: www.biochar-international.org (accessed on 26 February 2017).
29. Kang, S.; Shi, W.; Zhang, J. An improved water-use efficiency for maize grown under regulated deficit irrigation. *Field Crops Res.* **2000**, *67*, 207–214. [CrossRef]
30. Jalota, S.K.; Singh, S.; Chahal, G.B.S.; Ray, S.S.; Panigraghy, S.; Bhupinder-Singh; Singh, K.B. Soil texture, climate and management effects on plant growth, grain yield and water use by rainfed maize–wheat cropping system: Field and simulation study. *Agric. Water Manag.* **2010**, *97*, 83–90. [CrossRef]
31. *ISO 10390:2005 Soil Quality—Determination of pH*; ISO: Geneva, Switzerland, 2005.
32. Environmental Protection. *Testing of Soils. Determination of Organic Matter*; MSZ 21470-52:1983; Hungarian Standard Association: Budapest, Hungary, 1984.
33. ISO 10694:1995. *Soil Quality—Determination of Organic and Total Carbon after Dry Combustion*; ISO: Geneva, Switzerland, 1995.
34. Evaluation of Some Chemical Properties of the Soil. *Laboratory Tests. (pH Value, Phenolphtalein Alkalinity Expressed in Soda, Total Water Soluble Salt Content, Hydrolytic (y1 Value) and Exchangeable Acidity (y2 Value)*; MSZ-08-0206/2, 1978; Hungarian Standard Association: Budapest, Hungary, 1978. (In Hungarian)
35. *Determination of the Soluble Nutrient Element Content of the Soil*; MSZ 20135, 1999; Hungarian Standard Association: Budapest, Hungary, 1999.
36. ISO. *ISO 11261:1995 Soil Quality—Determination of Total Nitrogen—Modified Kjeldahl Method*; ISO: Geneva, Switzerland, 1995.
37. Environmental Testing of Soils. *Determination of Total and Soluble Toxic Element, Heavy Metal and Chromium (VI) Content*; MSZ 21470-50:2006; Hungarian Standard Association: Budapest, Hungary, 2006.
38. Anderson, J.P.E.; Domsch, K.H. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biol. Biochem.* **1978**, *10*, 215–221. [CrossRef]
39. Szili-Kovács, T.; Zsuposné Oláh, Á.; Kátai, J.; Villányi, I.; Takács, T. Correlations between biological and chemical soil properties in soils from long-term experiments. *Agrokém. Talajt.* **2011**, *60*, 241–254. [CrossRef]
40. Trouvelot, A.; Kough, J.L.; Gianinazzi-Pearson, V. Mesure du taux de mycorhization VA d'un système radiculaire. Recherches et methods d'estimation ayant une signification fonctionnelle. In *Physiological and Genetical Aspects of Mycorrhizae*; Gianinazzi-Pearson, V., Gianinazzi, S., Eds.; INRA: Paris, France, 1986; pp. 217–221.
41. Phillips, J.M.; Hayman, D.S. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **1970**, *55*, 158–161. [CrossRef]
42. Masto, R.E.; Kumar, S.; Rout, T.K.; Sarkar, P.; George, J.; Ram, L.C. Biochar from water hyacinth (*Eichornia crassipes*) and its impact on soil biological activity. *CATENA* **2013**, *111*, 64–71. [CrossRef]
43. Denmead, O.T.; Shaw, R.H. The effects of soil moisture stress at different stages of growth on the development and yield of corn. *Agron. J.* **1960**, *52*, 272–274. [CrossRef]
44. Jones, D.L.; Rousk, J.; Edwards-Jones, G.; DeLuca, T.H.; Murphy, D.V. Biochar-mediated changes in soil quality and plant growth in a three year field trial. *Soil Biol. Biochem.* **2012**, *45*, 113–124. [CrossRef]
45. Whitman, T.; Singh, B.P.; Zimmerman, A.R. Priming effects in biochar-amended soils: Implications of biochar-soil organic matter interactions for carbon storage. In *Biochar for Environmental Management: Science, Technology and Implementation*; Lehmann, J., Joseph, S., Eds.; Routledge: New York, NY, USA, 2015; pp. 455–488, ISBN 978-0-415-70415-1.
46. Cross, A.; Sohi, S.P. The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. *Soil Biol. Biochem.* **2011**, *43*, 2127–2134. [CrossRef]
47. Jien, S.H.; Wang, C.S. Effects of biochar on soil properties and erosion potential in a highly weathered soil. *CATENA* **2013**, *110*, 225–233. [CrossRef]
48. Tan, Z.; Lin, C.S.K.; Ji, X.; Rainey, T.J. Returning biochar to fields: A review. *Appl. Soil Ecol.* **2017**, *116*, 1–11. [CrossRef]

49. Von der Weid, I.; Alviano, D.S.; Santos, A.L.S.; Soares, R.M.A.; Alviano, C.S.; Seldin, L. Antimicrobial activity of *Paenibacillus peoriae* strain NRRL BD-62 against a broad spectrum of phytopathogenic bacteria and fungi. *J. Appl. Microbiol.* **2003**, *95*, 1143–1151. [CrossRef]
50. Mazzilli, S.R.; Kemanian, A.R.; Ernst, O.R.; Jackson, R.B.; Piñeiro, G. Greater humification of belowground than aboveground biomass carbon into particulate soil organic matter in no-till corn and soybean crops. *Soil Biol. Biochem.* **2015**, *85*, 22–30. [CrossRef]
51. Shepherd, M.A. Factors affecting nitrate leaching from sewage sludges applied to a sandy soil in arable agriculture. *Agric. Ecosyst. Environ.* **1996**, *58*, 171–185. [CrossRef]
52. Lehmann, J.; Pereira da Silva, J., Jr.; Steiner, C.; Nehls, T.; Zech, W.; Glaser, B. Nutrient availability and leaching in an archaeological Anthrosol and Ferralsol of the Central Amazon basin: Fertilizer, manure and charcoal amendments. *Plant Soil* **2003**, *249*, 343–357. [CrossRef]
53. Shepherd, J.G.; Buss, W.; Sohi, S.P.; Heal, K.V. Bioavailability of phosphorus, other nutrients and potentially toxic elements from marginal biomass-derived biochar assessed in barley (*Hordeum vulgare*) growth experiments. *Sci. Total Environ.* **2017**, *584*, 448–457. [CrossRef]
54. Quilliam, R.S.; Glanville, H.C.; Wade, S.C.; Jones, D.L. Life in the 'charosphere'—Does biochar in agricultural soil provide a significant habitat for microorganisms? *Soil Biol. Biochem.* **2013**, *65*, 287–293. [CrossRef]
55. Nguyen, T.T.; Marschner, P. Soil respiration, microbial biomass and nutrient availability in soil after repeated addition of low and high C/N plant residues. *Biol. Fertil. Soils* **2016**, *52*, 165–176. [CrossRef]
56. Brimecombe, M.J.; De Leij, F.A.; Lynch, J.M. The effect of root exudates on rhizosphere microbial populations. In *The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface*; Pinton, R., Varanini, Z., Nannipieri, P., Eds.; Marcel-Dekker, Inc.: New York, NY, USA, 2001; pp. 95–140, ISBN 0-8247-0427-4.
57. Ziadi, N.; Whalen, J.K.; Messiga, A.J.; Morel, C. Assessment and modeling of soil available phosphorus in sustainable cropping systems. *Adv. Agron.* **2013**, *122*, 85–126. [CrossRef]
58. Satyaprakash, M.; Nikitha, T.; Reddi, E.U.B.; Sadhana, B.; Vani, S.S. Phosphorous and phosphate solubilising bacteria and their role in plant nutrition. *Int. J. Curr. Microbiol. Appl. Sci.* **2017**, *6*, 2133–2144.
59. Parniske, M. Arbuscular mycorrhiza: The mother of plant root endosymbioses. *Nat. Rev. Microbiol.* **2008**, *6*, 763–775. [CrossRef] [PubMed]
60. Priyadharsini, P.; Muthukumar, T. Interactions between arbuscular mycorrhizal fungi and potassium-solubilizing microorganisms on agricultural productivity. In *Potassium Solubilizing Microorganisms for Sustainable Agriculture*; Meena, V.S., Maurya, B.R., Verma, J.P., Meena, R.S., Eds.; Springer: New Delhi, India, 2016; pp. 111–125, ISBN 978-81-322-2774-8. [CrossRef]
61. Adesemoye, A.O.; Kloepper, J.W. Plant–microbes interactions in enhanced fertilizer-use efficiency. *Appl. Microbiol. Biotechnol.* **2009**, *85*, 1–12. [CrossRef] [PubMed]
62. Hammer, E.C.; Balogh-Brunstad, Z.; Jakobsen, I.; Olsson, P.A.; Stipp, S.L.; Rillig, M.C. A mycorrhizal fungus grows on biochar and captures phosphorus from its surfaces. *Soil Biol. Biochem.* **2014**, *77*, 252–260. [CrossRef]