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Technical Note

Fourfold Increase in Pumpkin Yield in Response to Low-Dosage Root Zone Application of Urine-Enhanced Biochar to a Fertile Tropical Soil

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Abstract: A widely abundant and invasive forest shrub, *Eupatorium adenophorum*, was pyrolyzed in a cost-efficient flame curtain kiln to produce biochar. The resulting biochar fulfilled all the requirements for premium quality, according to the European Biochar Certificate. The biochar was either applied alone or mixed with fresh cow urine (1:1 volume) to test its capacity to serve as slow release fertilizer in a pumpkin field trial in Nepal. Treatments included cow-manure compost combined with (i) urine-only; (ii) biochar-only or (iii) urine-loaded biochar. All materials were applied directly to the root zone at a biochar dry matter content of 750 $kg \cdot ha^{-1}$ before seeding. The urine-biochar treatment led to a pumpkin yield of 82.6 $t \cdot ha^{-1}$, an increase of more than 300% compared with the treatment where only urine was applied, and an 85% increase compared with the biochar-only treatment. This study showed for the first time that a low-dosage root zone application of urine-enhanced biochar led to substantial yield increases in a fertile silt loam soil. This was tentatively explained by the formation of organic coating of inner pore biochar surfaces by the urine impregnation, which improved the capacity of the biochar to capture and exchange plant nutrients.

Keywords: biochar; organic fertilizer; organic coating; flame curtain pyrolysis; pumpkin; urine; root zone fertilizer application

1. Introduction

The majority of biochar field trials performed in the last decade used 10 to 50 $t{\cdot}ha^{-1}$ of fresh biochar [1–3]. Apart from some notable exceptions mostly in weathered and eroded tropical soils [4,5], the increases in crop productivity were lower than expected. A recent meta-analysis of 60 published biochar field trials [4] revealed that the average yield increase in crop production was around 18%. The authors of the study further concluded that positive changes in crop productivity might only be achieved when biochar application rates exceed 5 $t{\cdot}ha^{-1}$ [6]. However, cost-benefit calculations clearly showed that at current prices for industrially produced biochar (*i.e.*, 600–900 $Euros{\cdot}t^{-1}$) [7], the application of such large amounts of biochar is economically not viable for farmers [8].

The three biochar meta-analyses available to date [1,3,4] exclusively compared the effect of freshly produced biochar with that of the respective control, and did not account for composted or otherwise nutrient-enriched biochars. In many cases, it has been reported that application of freshly produced biochar may hinder a potential crop production enhancement or even reduce plant growth due to nutrient immobilization [9–12]. In order to eliminate or counteract the negative effects of applying freshly produced biochars, the enhancement of biochar with either organic or mineral nutrients has been suggested [13–16]. The most applied biochar nutrient functionalization process appears to be the blending with compost or a co-composting procedure [16,17]. In both cases, the porous biochar adsorptive surface sites are charged with nutrients [18,19], thereby stimulating microbial colonization [20], improving biochar surface reactivity through accelerated oxidative aging [21,22] and promoting dissolved organic carbon (DOC) adsorption and coating [18,23–25].

Field and pot trial results following the application of biochar-compost blends revealed substantial yield increases [26–29] and suggested that the total application amount of biochar can be reduced.

Kammann *et al.* [12] showed that co-composted biochar became highly enriched with nitrate, ammonium, phosphate and DOC, conceivably because of the entrapment of the dissolved nutrients during composting. Subsequent pot trials with the co-composted biochar manually extracted from the compost led to plant growth stimulation of *Chenopodium quinoa* of 305% *versus* −61% with untreated biochar compared with the unamended control. These results confirmed that organically enhanced biochars can substantially increase biomass production compared with fresh biochar.

The combination of biochar with compost and also with urine or other nutrient-rich organic liquids has been suggested to produce an effective biochar-based fertilizer [15,30]. Field applications of nutrient-enhanced biochars can potentially reduce the necessity to apply mineral fertilizers, can benefit organic farmers, or farmers from low-income regions, and can improve biochar related cost-benefit ratios. Moreover, it is very likely that combining urine and biochar would reduce adverse excess nutrient effects of sole urine applications, such as nitrogen leaching or N_2O greenhouse gas emissions [31].

Farmers in Switzerland, Germany and Austria already use biochar as an additive in animal bedding and liquid manure treatment [32], and many proponents of permaculture use biochar as a soaking agent for separating toilets, both with subsequent uses of the organic nutrient-enhanced biochar as soil amendments. However, to the best of our knowledge, no scientific study has yet been undertaken to investigate the fertilizing properties of urine-enriched biochar.

Another way forward to reduce the biochar application rate per hectare is the modification of the application method. While most biochar applications described so far consisted of homogenous spreading and ploughing over the whole field plot, conservative farming practices propose to apply soil amendments in a more concentrated fashion and close to the roots, e.g., in the hoe basins or rip lines where cultivation occurs [33–35]. Blackwell *et al.* [36] tested banded biochar application on dry land wheat production and concluded that the banding application of biochar at low application rates of 1 $t{\cdot}ha^{-1}$ can provide significant positive effects on yield while reducing fertilizer requirements. Graves [37] described and compared agro-mechanical methods of deep banding and root zone injection, reporting positive results. In Zambia, biochar produced from maize cobs was applied at only 4 $t{\cdot}ha^{-1}$ in hoe-dug pits before seeding maize; this increased harvests by up to a factor of four in a weathered acidic sandy soil [4]. However, the biochar in the Zambian trial was applied fresh from production and was not enhanced with organic nutrients; it can therefore be assumed that the application rate might be further decreased when the root zone application is performed with nutrient-enhanced biochar slurry [38,39].

A third way to improve the biochar related cost-benefits for agricultural uses (at least for small and subsistence farmers) would be that farmers produce (on the farm) their own biochar from residues such as straw, husks, animal feed left over, shrubs, cuttings and prunings at a small scale by using low-cost but high quality biochar kilns with clean combustion. Farmers can thus combine biochar production with on-farm waste management, biomass heat generation and on-site organic fertilizer production [7], reducing greenhouse gas emissions from uncontrolled decomposition and open burning. With the Kon-Tiki flame curtain kilns developed in 2014 in Switzerland [40], a technology that spread rapidly by open source transfer to many farmers in both developing and developed countries [41], a first step towards economic farm-scale biochar production seems to have been initiated.

In short, the principle of the flame curtain pyrolysis consists of pyrolyzing biomass layer by layer in a conically formed metal kiln or soil pit. A fire is started in the kiln, and the burning embers spread to form a first layer on the bottom of the kiln. A thin layer of biomass is then added on top of the embers, which heats rapidly and starts to outgas. The rising pyrolysis gas is caught in the flames and reacts with combustion air entering the kiln from the top. Below the flames, the biomass carbonizes since the oxygen is consumed above. When ash appears on the outside of the carbonizing biomass, the next layer of biomass is homogenously spread on top. Convective and radiant energy from the flames above and from the hot pyrolyzing layers below heat the fresh biomass layer, which starts to pyrolyze.

The biochar below the upper pyrolysis layer is safeguarded from oxygen by the fire itself. The combustion zone forms a flame curtain that protects the underlying biochar from oxidizing and cleanly burns all pyrolysis smoke and gases as they pass through this hot fire front. The manual layering of biomass is repeated until the metal kiln or soil pit is filled. The pyrolysis process is then actively ended by quenching with water or a nutrient solution (urine or dissolved fertilizer) or by snuffing with a soil layer. One run of an average sized kiln with an upper diameter of 1.5 m produces 800 liters of biochar in about 2 h.

In 2015, a pumpkin field trial was established at eight farms in Dhading, Nepal, where a *Eupatorium* biochar made by flame curtain pyrolysis was tested in combination with cow urine. Three soil amendments were compared: urine-only, biochar-only, and urine-soaked-biochar. We investigated the following three hypotheses: (1) application of biochar to the root zone of pumpkins reduces the application rate necessary to achieve positive yield effects to below 1 $t \cdot ha^{-1}$; (2) mixing of biochar with cow-urine improves fertilizer efficiency of urine; and (3) applying urine-enhanced biochar at low doses ($<1\ t \cdot ha^{-1}$) to the root zone will lead to substantial increases in crop productivity even in fertile soils, comparable to yields achieved in fully fertilized conventional farms.

2. Experimental Section

2.1. Experimental Site

The eight field sites were located in the Nalang village in the Dhading district of Nepal (27°50′ N, 84°50′ E), which is situated on the western bank of the Thopal Khola River at an average altitude of 450 m above mean sea level. The climate is subtropical with a single rainy season between mid-June to mid-September. The annual mean temperature and annual precipitation at the nearby meteorological station of Dhading Besi are 22.8 °C and 2329 mm, respectively. During the period of the experiment from January to May 2015, precipitation was 360 mm with an average temperature of 25.1 °C, both corresponding to the normal climate pattern of this period and region.

The experiment was laid out with an identical set-up at 8 different field sites, all within a distance of 2 km from the Nalang village. At all field sites, the soils were classified as silt loam. The soils were slightly acidic with pH values ranging between 4.5 and 6.7. With soil organic matter (SOM) contents ranging from 1.9% to 5.5%, cation exchange capacities (CEC) between 36 and 65 $meq \cdot 100\ g^{-1}$, and considerable available and exchangeable plant nutrients, five of the soils were considered as fertile and the other three as rather fertile (Table 1). All eight farms participating in this trial usually practice organic farming methods. No mineral fertilizer, herbicides or chemical pesticides were used either during the trial or in the five years before the trial.

2.2. Experimental Setup

The eight field trials were established on a long terrace. As the terraces were built into steep slopes of the Thopal Khola River and were only 2 to 2.5 m wide, the pumpkins were grown in a long single row with an interplant distance of 1.90 m. Each trial consisted of three, four or five randomized blocks, with each block containing three treatment plots covering an area of 3.8 m^2. Each treatment plot contained one pumpkin plant (Figure 1). Plant density was 2630 $plants \cdot ha^{-1}$. Four farmers conducted the trial with five repetitions, three farmers with four repetitions and one farmer with only three repetitions due to the size limitation of their respective trial terraces, which amounted in a total of 104 plots.

The three treatments were: (A) 10.5 t·ha^{-1} compost + 6.3 m^{3}·ha^{-1} cow urine (referred to hereafter as "urine-only"); (B) 10.5 t·ha^{-1} compost mixed with 0.75 t·ha^{-1} (dry matter (DM)) biochar without cow urine (referred to hereafter as "biochar-only"); (C) 10.5 t·ha^{-1} compost + 0.75 t·ha^{-1} (DM) biochar macerated in 6.3 m^{3}·ha^{-1} cow urine (referred to hereafter as "urine-biochar").

Table 1. Analytical parameters of the eight trial soils.

Analyzed Parameters	**Field Site Location**								**Test Method/Instrument**
	Nirmala Timilsina	Bimala Lamsal	Uma Aryal	Sani Aryal	Sita Lamsal	Tank Timilsina	Ambika Aryal	Anisa Kattel	
pH	6.71	4.55	4.9	6.42	4.48	5.64	5.94	5.99	Measured in H_2O suspension
Soil Organic Matter (SOM)%	5.37	2.04	1.93	5.54	2.37	3.06	2.74	2.62	Weight loss on ignition at 360 °C
Total Nitrogen (mg·kg^{-1})	2093	1820	1512	2002	2044	2072	2184	1960	Kjeldahl method
Available Phosphorus (mg·kg^{-1})	235.05	143.3	107.5	120.45	124.6	308.4	130.8	308.4	Olsen P-Method
Exchangeable Potassium (K), (mg·kg^{-1})	135.7	68.98	77.29	502.8	69.83	257.3	160	98.98	Ammonium acetate followed by atomic absorbtion spectroscopy
CEC (meq 100 g^{-1})	48.1	65.4	53.8	59	59.4	36.2	52.6	40	Ammonium acetate extraction
Exchangeable Calcium(Ca), (mg·kg^{-1})	1154	171.7	305	800	190	1083	1133	950	Ammonium acetate extraction
Exchangeable Magnesium (Mg), (mg·kg^{-1})	411	97.27	136.2	421	96.53	415.3	459.1	433.1	Ammonium acetate extraction
Exchangeable Sodium (Na), (mg·kg^{-1})	32	47.66	19.16	75.87	27.73	15.26	44.81	9.42	Ammonium acetate extraction
Clay (%)	7	18	14	8	14	22	24	13	Hydrometer method
Sand (%)	27.27	4.82	5.59	15.96	16.22	13.31	13.29	35.73	
Silt (%)	65.73	77.18	80.41	76.04	69.78	64.69	62.71	51.27	
Texture class	Silt Loam	Silt Loam	Silt Loam	Silt Loam	Silt Loam	Silt Loam	Silt Loam	Silt Loam	

Analytical parameters and methods of the eight trial soil samples; each soil sample was mixed from 12 randomly distributed soil cores collected from a depth of 2 to 20 cm before trial establishment.

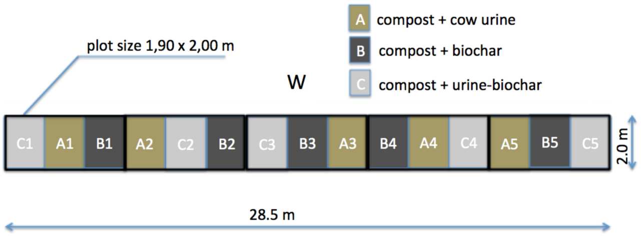

Figure 1. Trial set-up. Notes: Experimental set-up consisting of five randomized blocks. Each plot contained one pumpkin plant. The same set-up was used at each trial location, with three to five randomized blocks.

The field trials were set-up between 27 January and 8 February 2015. Before seeding, 40-cm deep planting pits with a diameter of about 35 cm were dug. According to farmer practice, 4 kg of cow manure compost (10.5 t/ha) was applied to each planting hole of each treatment. In treatment A, the compost was hand-mixed with some soil and then covered with 5 cm topsoil to close the planting pit. Subsequently, 2.4 l of fresh cow urine (6.3 $m^3 \cdot ha^{-1}$) was mixed at a ratio of 1:5 with water to avoid cauterization, and then poured over the planting area of each treatment plot, except for a small seeding ring of 10 cm diameter in the middle of the planting area. As urine rapidly penetrates the soil to a depth of 40 cm [42], it was decided to apply the water-mixed urine on the top and not at the bottom of the planting pit to avoid a too rapid downward flow and leaching of the nutrients to deeper soil horizons, which would then be out of the reach of the pumpkin roots. For each plot of treatment B, 290 g (DM) of production fresh biochar (0.75 $t \cdot ha^{-1}$) was mixed with the compost and some soil. For each plot of treatment C, 290 g (DM) of biochar (0.75 $t \cdot ha^{-1}$) was mixed with 2.4 L of fresh cow urine (6.3 $m^3 \cdot ha^{-1}$), which corresponds roughly to a volume ratio of 1:1. The urine-biochar slurry was macerated for at least 1 h and was subsequently mixed with the compost and some soil inside the planting pit. All planting pits of treatments B and C were then covered with 5 cm of top soil to close the planting pit, as was done for treatment A.

On top of each planting pit, two seeds of the local pumpkin (*Cucurbita maxima L.*) variety *new grace*, locally called "*Maddle*", were applied, covered with 2 cm of topsoil and then watered with 5 L of water. One week after germination, the weaker of the two seedlings was weeded. In all plots, at least one of the two plants emerged and none of the plants withered during the trial period. All plots were equally irrigated during the first two drier months following local farmer practice. The plots were hand weeded; no supplemental fertilizer or pesticides were applied until harvest. Each farmer maintained the trial during the trial period, while two designated lead farmers (Sita Lamsal and Anisa Kattel) visited each trial at least once a week to ensure equal treatment.

The plots were harvested between 23 and 29 May 2015. The two lead farmers were present during the harvest of each trial and measured the weight of the harvested pumpkins using the same scales for each trial.

2.3. Biochar Characterization

The biochar was produced in a Kon-Tiki flame curtain pyrolysis kiln [40] installed in the backyard of one of the participating farmers. The feedstock for the biochar production was *Eupatorium adenophorum*, a very frequently occurring invasive forest shrub species that local people call "ban mara" (*i.e.*, forest killer) [43]. Due to the dry season, the shrubs were mostly dry and were pyrolyzed with all branches and leaves at 680–700 °C. After a pyrolysis time of about one hour and having produced roughly 0.5 m^3 of biochar, the biochar was quenched with water. As the *Eupatorium* branches are thin and the resulting biochar friable, no milling and only slight crushing was necessary. The average particle size was estimated to be below 10 mm. The biochar had a pH of 9.8, a carbon content of 72% and an H/C_{org} molar ratio of 0.2. With a specific surface area (BET) of 215 $m^2 \cdot g^{-1}$ and a specific weight of only 120 $g \cdot L^{-1}$, this very lightweight, low-density biochar had a high water-holding capacity 6.5 times its own weight. All analytical parameters were well within the thresholds for premium quality of the European Biochar Certificate [44]. At a biochar application rate of 750 $kg \cdot ha^{-1}$, the nutrient amounts applied with the biochar were negligible except for potassium. At 2.8% (*w*/*w*) potassium, the application of 750 $kg \cdot ha^{-1}$ biochar corresponded to 21 $kg \cdot ha^{-1}$ potassium. Further physical and chemical properties of the biochar and the respective analytical methods are summarized in Table 2.

Table 2. Analytical parameters of the biochar.

Parameter	Unit	in Fresh Matter	in Dry Matter
Density	$kg \cdot m^{-3}$	778	120
Specific surface (BET)	$m^2 \cdot g^{-1}$	-	215
Ash 550 °C	mass-%	3.4	21.9
Hydrogen	mass-%	0.21	1.33
Carbon	mass-%	11.1	72
Nitrogen	mass-%	0.08	0.54
Oxygen	mass-%	0.6	4.0
Carbonate CO_2	mass-%	<0.4	2.24
Organic carbon	mass-%	11.1	71.4
H/C org. (molar)		0.23	0.22
O/C (molar)		0.04	0.042
pH		9.8	-
Electric conductivity	$\mu S \cdot cm^{-1}$	9090	-
Salt content	$g \cdot kg^{-1}$	8.25	53.7
Phosphorous	$mg \cdot kg^{-1}$	-	3700
Magnesium	$mg \cdot kg^{-1}$	-	12,000
Calcium	$mg \cdot kg^{-1}$	-	17,000
Potassium	$mg \cdot kg^{-1}$	-	28,000
Sodium	$mg \cdot kg^{-1}$	-	520
Iron	$mg \cdot kg^{-1}$	-	6000
Silicium	$mg \cdot kg^{-1}$	-	34,000
Sulfur	$mg \cdot kg^{-1}$	-	860
Naphthalene	$mg \cdot kg^{-1}$	-	2.0
Phenanthrene	$mg \cdot kg^{-1}$	-	0.8
Anthracene	$mg \cdot kg^{-1}$	-	0.2
Fluoranthene	$mg \cdot kg^{-1}$	-	0.6
Pyrene	$mg \cdot kg^{-1}$	-	0.5
Benzo (a)pyrene	$mg \cdot kg^{-1}$	-	<0.1
SUM polycyclic aromatic hydrocarbons (EPA 16)	$mg \cdot kg^{-1}$	-	4.9

Analytical methods following the European Biochar Certificate [44].

2.4. Cow Urine and Compost Nutrient Analyses and NPK Application Rates

The cow urine was collected fresh one day before the set-up of each respective trial from the same cows of the same stable. One freshly mixed sample was transported in a cool box to the soil laboratory of the Aquatic Ecology Centre at Kathmandu University in Dhulikhel for analysis. The total nitrogen (TN), phosphate (P_2O_5) and potassium (K) content of the cow urine were 9.5 $g \cdot L^{-1}$, 0.35 $g \cdot L^{-1}$, and 9.2 $g \cdot L^{-1}$, respectively, corresponding to approximately 60 $kg \cdot ha^{-1}$ TN, 2 $kg \cdot ha^{-1}$ P_2O_5, and 58 $kg \cdot ha^{-1}$ K application rates, respectively, confirming the average nutrient contents of cow urine known from the literature [45–48]. Urinary P excretion in ruminants is generally considered minimal [49] and is nearly 100 times lower than in solid cow manure [48]. The pH of the cow urine sample was 7.5.

The cow manure compost that was initially mixed with approximately 30% straw was matured for at least five months, and was considered as mature (complete degradation of straw, no bad odors). The compost for all trials was collected from the same compost pile. The cow manure compost that was used in equal amounts in each plot was well matured and had a total nitrogen, phosphate and potassium content of 14 $g \cdot kg^{-1}$ (DM), 7 $g \cdot kg^{-1}$ (DM) and 18 $g \cdot kg^{-1}$ (DM), respectively. Considering that only 15% of the compost N, and 30% of each of the compost phosphate and potassium are in a plant-available form in the first year [50–52], the amount of plant available N, P_2O_5 and K applied with the compost was a maximum of 22 $kg \cdot ha^{-1}$, 22 $kg \cdot ha^{-1}$, and 57 $kg \cdot ha^{-1}$, respectively. Although the compost for all trials and treatments was collected from the same pile, the substrate is naturally inhomogeneous and the 4 kg of compost that was applied to each planting pit can thus not be considered as a representative sample, but rather as a specimen [53]. The amount of plant available nutrients applied with the compost and urine in each respective planting pit can thus only be given as an approximation.

The NPK input rate per hectare corresponded to approximately 82:24:115 for the urine-only treatment, 22:22:78 for the biochar-only treatment and 82:24:136 for the urine-biochar treatment.

2.5. Statistical Analysis

Statistical analyses of the complete data set of all eight field trial sites were conducted using the libraries lme4 and multcomp in the statistical software package "R" (version 3.2.1) [54]. We used linear mixed effects models (lmer) with site-specific random effects (eight sites). The categorical explanatory variable (fixed effects) included the three treatment levels: urine-only, biochar-only and urine-biochar. Likelihood ratio tests were used to simplify the fixed effects structure of the models (fitted by maximum likelihood (ML)). The best model was refitted on the basis of restricted maximum likelihood (REML) and the estimated effects (including standard error) were calculated using general linear hypothesis testing (glht in multcomp). Residuals and predicted random effects were plotted (histograms and QQ normal plots) to assess normality and potential outliers.

For the statistical analysis of the individual sites, normality was tested with the Shapiro-Wilk test and homogeneity of variances with Levene's test. Data not following the normal distribution were log-transformed. Significance of treatments ($p < 0.05$) was tested by ANOVA, and differences between treatments were subsequently tested using Tukey's multiple comparison test.

3. Results and Discussion

In the eight field trials, the pumpkin yield increased by 306% ($p < 0.0001$) in the urine-biochar treatment compared with the treatment where only urine was applied (~31 and ~8 kg·plant^{-1}, respectively), and by 85% ($p < 0.0001$) compared with the biochar-only treatment (~17 kg·plant^{-1}). The biochar-only treatment caused a significant increase of 119% ($p = 0.0033$) compared with the treatment where only urine was applied (Figure 2). All treatments received the same amount of compost as the root zone application.

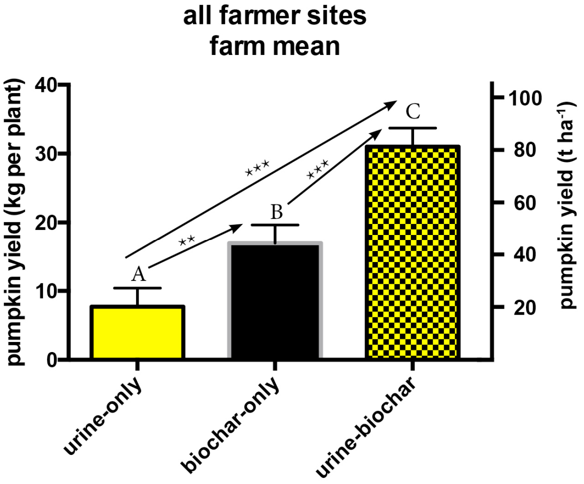

Figure 2. Mean pumpkin yield of all farmer sites. Notes: Mean pumpkin yield of all farmer sites in kg per plant and t·ha^{-1}. The statistic analysis was calculated with the linear mixed effects models (lmer), including the site-specific random effects of the eight sites. Codes of significance are *** for $p < 0.001$ and ** for $p < 0.01$. The yield in t·ha^{-1} was calculated with a plant density of 2630 plants·ha^{-1}.

Individual analysis of the eight sites revealed that with the exception of the Sita Lamsal trial location, all sites had significant ($p < 0.05$) trial results (Table 3); the yields in the urine-biochar treatment were consistently significantly higher ($p < 0.05$) than those in the urine-only treatment and mostly significantly higher ($p < 0.05$) than the treatments with biochar-only (four out of seven).

Table 3. Mean pumpkin yield of the eight individual trials.

Farmers' Names	*N*	*p*	Urine-Only	Biochar-Only	Biochar-Urine
Nirmala Timilsina	4	0.0093	11.0 ± 6.78^{a}	18.75 ± 6.13^{ab}	26.75 ± 2.63^{b}
Bimala Lamsal	5	0.0026	9.4 ± 3.72^{a}	16.6 ± 8.08^{a}	27.4 ± 6.43^{b}
Uma Aryal	5	0.0493	9.0 ± 3.94^{a}	12.6 ± 8.23^{ab}	24.25 ± 11.84^{b}
Sani Aryal	3	0.004	5.67 ± 0.58^{a}	14.33 ± 4.73^{a}	25.33 ± 5.69^{b}
Sita Lamsal	4	ns	6.5 ± 7.55^{a}	25.0 ± 24.06^{a}	36.0 ± 29.02^{a}
Tank Timilsina	4	0.0319	4.0 ± 3.74^{a}	12.75 ± 8.14^{ab}	19.0 ± 7.17^{b}
Ambika Aryal	5	0.0013	3.8 ± 3.63^{a}	12.4 ± 8.56^{a}	32.0 ± 13.11^{b}
Anisa Kattel	5	0.0069	12.6 ± 13.2^{a}	24.6 ± 14.14^{a}	55.8 ± 24.26^{b}

Mean pumpkin yield of the eight individual trials in kg per plant with standard deviation; values with different letters are significantly different (at $p < 0.05$) in same line.

The total yields as well as the percentage yield increases of the urine-biochar and biochar-only treatments compared with the urine-only treatment varied strongly between the individual sites (Table 3). While the percentage yield increase of urine-biochar compared with urine-only treatments varied between 116% (Uma Aryal) and 742% (Ambika Aryal), the percentage yield increase of the urine-biochar and biochar only treatments varied between 43% and 158%. At all field sites, the soils were slightly acidic though half of the trial location had soils within the optimal soil pH for pumpkin cultivation (*i.e.*, 6 to 6.5) [55,56], whereas the other half of the sites had soil pH values up to 1.5 units below the optimal range. However, no correlation was found between the soil pH of a given trial location and its total yield or percentage yield increase (see Tables 1 and 3). Other soil parameters such as soil organic carbon (SOC), CEC or nutrient content did not provide any functional relationship with the total yield or yield increase variations of the individual trial locations. It is therefore hypothesized that natural variation, field history (terrace construction, erosion, fertilization, preceding crops and crop residues), and influence of agroforestry elements were responsible for the variation in the total yield and yield increases.

The average yield of giant pumpkins in optimized conventional pumpkin production with controlled fertigation is 20 to 70 $t \cdot ha^{-1}$ depending on cultivar, soil, climate and plant density [44–46]. The average yield in the urine-only treatment was 20.4 $t \cdot ha^{-1}$, *i.e.*, at the lower end of conventional farming standards, but by accounting for the low, sub-optimal one-dosage liquid fertilization at the time of seeding, the yield is considerable and confirms a rather good basic fertility of the trial plot soils. While the 44.6 $t \cdot ha^{-1}$ yield obtained in the biochar-only treatment has to be considered as fairly high compared with optimized conventional farming, the average yield of 82.6 $t \cdot ha^{-1}$ that was reached in the urine-biochar treatment even exceeded the expected yield in optimized conventional pumpkin production (Figure 2).

The nitrogen inputs of urine-only and urine-biochar treatments (82 $kg \cdot ha^{-1}$) are both at the lower end of the industrial fertilizer recommendations for pumpkins, which are 80–160 $kg \cdot ha^{-1}$ [57–59]. With respect to phosphate (24 $kg \cdot ha^{-1}$) and potassium (115 $kg \cdot ha^{-1}$ for urine-only and 136 $kg \cdot ha^{-1}$ for urine-biochar), both urine treatments have to be considered as suboptimal compared with the fertilizer recommendations, which are 30–70 $kg \cdot ha^{-1}$ P_2O_5, and 220–300 $kg \cdot ha^{-1}$ K [57–59].

In the urine-only treatment, the fresh cow urine was mixed in a 1:5 ratio with water and was applied on top of the planting pits just before laying the seeds. It is unlikely that cauterization or other toxic effects of the urine caused the lower yields in this treatment as all seeds germinated correctly and the initial growth was normal. Wachendorf *et al.*, showed in a lysimeter trial with ^{15}N-labeled cow urine that an average of 60% of the urine N leached to the subsoil within one year in a temperate climate [60]. By considering the sub-tropical climate at the trial locations with 360 mm rain during the trial period, it is conceivable that approximately half of the urine-applied N in the urine-only plots leached out before the pumpkin roots had grown sufficiently to take up the nutrients. The remaining plant available N was apparently still sufficient to allow consistent growth compared with conventional pumpkin cultivation but was certainly sub-optimal in comparison to the other two treatments. To improve the nutrient efficiency of the urine, the urine-water mix could have been portioned into several dosages and applied regularly during the growing season. However, fertigation practices were not the subject of this trial as we intended to investigate whether mixing the same amount of cow urine with biochar and its subsequent application to the root zone would improve the nutrient efficiency and provide an organic slow release fertilizer that can be applied in the form of one initial dose.

The P_2O_5 and K inputs are slightly below the fertilizer recommendations but as both nutrients are less mobile than the applied N [61–63], a larger part than in the case of N can be taken up by the cultivated plant. It can, however, not be excluded that the rather suboptimal dosages of K and P_2O_5 were also limiting factors in the urine-only treatment.

Due to the surprisingly strong growth of the pumpkins plants in the urine-biochar treatment, the plot size per plant tended to be too small. To avoid shading of the adjacent plots, especially of the slower growing urine-only treatments, pumpkin sprouts growing too long were led to the lower lying terraces. Some shading effects, however, cannot completely be excluded and could have slightly influenced the differences between the treatments.

The nutrient input of the biochar-only treatment was very low compared with fertilizer recommendations and with the other treatments. In fact, it provided only about one quarter of the recommended fertilizer rate of N and K. It is all the more remarkable that the yield of the biochar-only treatment was more than double compared with the urine-only treatment, which had a much closer-to-optimal NPK application quantity and ratio. Most of the nutrients that were taken up by the pumpkin plants in the biochar-only treatment must thus have come from the compost and soil reserves. It is possible that the biochar stimulated the mineralization of the soil and compost nutrients and/or stimulated their uptake by the plants. The latter may partially be explained by the high water holding capacity of the biochar that was mixed to the compost and applied to the root zone of the pumpkin plants. Having a water holding capacity of $6.5\ g\cdot g^{-1}$, the 290 g (DM) of *Eupatorium* biochar can, when applied into the root zone of each plant, store approximately 1.9 L of extra water compared with the urine-only treatment. As the trials were not regularly watered by drip irrigation, but irregularly by rain or water buckets, the extra reserve of nearly 2 L of water in the root zone and the compost-nutrient deposit below each pumpkin plant could have resulted in an important difference.

It is likely that the biochar mixed with the compost in the root zone captured compost nutrients dissolved during rain events and irrigation, as shown by Kammann *et al.* [12] and others [64–67], thus likely reducing nutrient leaching, gaseous losses and/or immobilization (not measured here). Kammann *et al.* [12] further proved via repeated KCl extractions, that organic plant nutrients captured by biochar remain mostly plant available, which may partially explain the improved nutrient status of the pumpkin plants, compared with the non-biochar amended control treatment. It cannot be ruled out that the biochar delivered more micro-nutrients, but considering that urine and compost both contain all essential micro-nutrients [68], it is unlikely that the extra micro-nutrients delivered by the biochar would explain the high increase in pumpkin yield compared with the urine-only treatment.

As the pH of half of the trial soils was lower than the optimum range for pumpkin cultivation, it might be hypothesized that the pH increase in the root zone due to the concentrated application of the alkaline biochar (pH 9.8) led to improved plant growth conditions [2,69–71]. However, no correlation existed between soil pH and yield stimulation among the 8 sites or between the three sites with pH below 5 and those above this value (Tables 1 and 3). We therefore conclude that the influence of local pH alteration in the root zone cannot, or only to a minor extent, explain the strong differences in yield. Further biochar effects in the root zone potentially amplified by the concentrated deposit application might be expected for redox potential [71,72], CEC [73,74] and microbial activity [75] although this is outside the scope of this study and clearly needs more research.

The main potential biochar effects described for the biochar-only treatment (capturing and improved mineralization of compost nutrients, improved water holding ability, pH effect and increased CEC) are likely the same as for the urine-biochar treatment. The 85% increase in pumpkin yield of the urine-biochar treatment compared with the biochar-only treatment must therefore be explained by further interaction effects of the cow-urine with the biochar. While a major part of the urine without biochar most probably leached out of the upper soil, the initial mixing of the urine with biochar retained nutrients in the porous biochar system [12,76,77].

Urine is the result of ultrafiltration of the blood plasma in the kidneys. It contains ions, amino acids, enzymes, serpins, adhesion molecules, hundreds of different small proteins and a multiple of other organic molecules with a maximal molecular diameter of 4.4 nm, as well as cell debris and nuclei [78]. The naturally ultra-filtrated ions and organic molecules can penetrate the microporous structure of the biochar and even enter into the nanopore system (as nanopores are mostly larger than the aforementioned average molecular diameter) by water dynamics [79]. The organic compounds provided by the urine can be adsorbed onto the polyaromatic biochar carbon surfaces by (1) charge assisted hydrogen bonds, (e.g., H-bonds between the electron-rich, proton-acceptors in the urine-contained organic groups and the proton-donors on biochar surface sites, or, vice versa, between the electron-rich biochar aromatic systems and the proton-donors in the organic systems from the added urine); (2) water bridging (*i.e.*, water bridges between the solvation water of the organic and inorganic urine constituents and the hydrophilic systems, such as inorganic ashes and oxygenated organic functions, on biochar surfaces); and (3) Van der Waals interactions (*i.e.*, interactions among the hydrophobic moieties of the urine added organic molecules and the polyaromatic organic parts of biochar) [80–84]. It is hypothesized that the small organic molecules of the urine form an "organic coating" on intra-porous biochar aromatic carbon surfaces [80]. Subsequently, anions like nitrate or phosphate, as well as cations like ammonium, can be bound via water bridges to the organic coating of the biochar surfaces [12,79,81,84,85], thereby becoming reversibly sorbed. Urine-impregnated biochar may thus evolve into a new hybrid material with its carbon lattice working as a high surface carrier that may serve as a main reactive interface for nutrient capture and exchange with soil, soil biota and plants.

More experiments and research are clearly needed to further develop and prove the biochar-organic-mineral interaction hypothesis and to design the methods for safe implementation in agricultural and industrial practices. From an agronomic point of view, the most important questions to investigate are (1) which type of biochar is best suited for organic coating (feedstock, pyrolysis temperature, post pyrolysis oxygenation, quenching); (2) the optimal urine-to-biochar ratio depending on the type of biochar; (3) the technique, temperature and duration of the biochar impregnation with urine before soil application; and (4) nutrient leaching (respective retention), bioavailability, and effective fertilizer efficiency of urine impregnated biochars compared with urine use without biochar or other porous carriers like zeolite. Naturally, many other liquid organic fertilizers may work as well; however, urine (animal and human) has at least two distinct advantages: (1) it is ultra-filtrated and can thus penetrate even the nanopores of biochar; (2) it is a cheap ubiquitous waste product.

4. Conclusions

The biochar made from an invasive forest shrub and produced at low cost by farmers themselves using a flame curtain pyrolysis kiln was of excellent quality, qualifying for premium quality of the European Biochar Certificate. It was demonstrated that very low application amounts of biochar (below 1 $t \cdot ha^{-1}$) improved pumpkin yield when applied in concentrated form to the root zone alongside compost. Mixing the biochar with cow urine and applying the urine-biochar slurry to the root zone considerably improved the pumpkin yield compared with the treatments where biochar was applied without urine, or urine without biochar.

The pumpkin yield increases obtained in the urine-biochar treatments and the harvest results exceeded even those of optimized conventional farming, demonstrating that organic enhancement of biochar combined with concentrated root zone application can improve plant nutrition and hence crop productivity even at very low dosages of biochar on a per-hectare basis. Contrary to the conclusions in other studies [1,36], the described and demonstrated methods are not only efficient on weathered and degraded soils in suboptimal agronomic systems but may have the potential to develop into a new, long-term perspective for more sustainable, climate-positive and nutrient efficient agriculture on already fertile soils, particularly in organic farming systems.

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Author Contributions

Hans Peter Schmidt and Bishnu Hari Pandit conceived, designed and set up the experiments. Bishnu Hari Pandit organized and maintained the trial and collected the data. Hans Peter Schmidt, Vegard Martinsen, and Claudia I. Kammann analyzed the data. Hans Peter Schmidt, Bishnu Hari Pandit, Gerard Cornelissen, Pellegrino Conte and Claudia I. Kammann wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

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