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Abstract: Biochar addition to compost is of growing interest as soil amendment. However, little is known about the evolution of material properties of biochar-compost mixtures and their effect on plants after exposure to physical weathering. This study aimed to investigate the physico-chemical characteristics of fresh and weathered biochar-compost mixtures, their biological stability and their effect on ryegrass growth. To this end, we used the contrasting stable isotope signatures of biochar and compost to follow their behavior in biochar-compost mixtures subjected to artificial weathering during 1-year of incubation. We assessed their impact on ryegrass growth during a 4-week greenhouse pot experiment. Weathering treatment resulted in strong leaching of labile compounds. However, biochar-compost interactions led to reduced mass loss and fixed carbon retention during weathering of mixtures. Moreover, weathering increased carbon mineralization of biochar-compost mixtures, probably due to the protection of labile compounds from compost within biochar structure, as well as leaching of labile biochar compounds inhibiting microbial activity. After soil application, weathered mixtures could have positive effects on biomass production. We conclude that biochar-compost interactions on soil microbial activity and plant growth are evolving after physical weathering depending on biochar production conditions.

Keywords: biochar; compost; isotopic signature; carbon mineralization; plant growth

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1. Introduction

According to the last report of the Intergovernmental Panel of Climate Change (IPCC), global temperatures have increased by 1 °C above pre-industrial levels due to human activity [1]. Further increase should be limited to 1.5 °C in order to prevent dangerous climate change. To achieve this goal, active carbon dioxide removal from the atmosphere and its storage is needed [1]. Soil carbon sequestration and biochar application to soils may be used for this purpose. As negative emission technologies (NETs), their implementation may be able to achieve long-term carbon sequestration and may have advantages over the other NETs related to their effect on land use, water use and energy requirement [2].

Soil carbon (C) sequestration may be enhanced by the addition of organic amendments. While organic residues such as plant material or manure are usually transformed into amendments through composting, they may also be the feedstock for biochar production [3]. Biochar is a solid pyrolysis product intended to be used as soil amendment

[4]. It is mainly composed of aromatic C and has favourable properties such as large porosity and surface area in addition to high cation exchange capacity, depending on feedstock, pyrolysis conditions and particle size [5–7]. Biochar is known to improve soil properties such as water retention under drought conditions [8], and soil aggregate stability and porosity [9,10]. Due to its low nutrient content, biochar should be combined with nutrient additions through mineral fertilizers, compost and/or growth promoting micro-organisms to further increase its beneficial effects on plant growth when applied to soil [3]. On the other hand, compost is rapidly mineralized after soil application and its carbon sequestration potential may be enhanced by combination with organic and inorganic additives [11]. Mixtures of both materials may therefore be an innovative practice, leading to more efficient soil amendment as compared to their single use.

Biochar combination with other organic amendments may have synergistic effects on organic C retention, which were attributed to physical protection of compost by its occlusion into aggregates or adsorption on biochar surface [12–14]. Other studies found that biochar and mature compost mixtures induced a negative priming effect [15] or a neutral effect [16] on C mineralization when compared to application of compost. Soil addition of biochar-compost mixtures was shown to promote plant growth, biomass accumulation, yield and to improve soil properties such as water holding capacity [17–22]. Yet the synergistic effects of freshly applied biochar-compost mixtures on plant growth and performance are still under debate [23]. Indeed, application of fresh biochar-compost mixture has been found to have neutral [18] or even antagonisms effects [23]. This may be due to release of toxic compounds contained in the biochars' labile fraction [24–27] or to low availability of nutrients due to the biochars' high sorption capacity [23].

When applied to the field and exposed to weathering, the mixture effects may prevent carbon and nitrogen losses as compared to the single use of compost and biochar [28]. Physical weathering may increase the biological stability of biochars and reduce their priming effect on native SOM mineralization [29]. Moreover, weathering may change the biochar structure [30] and its effects on soil properties [8]. These effects may also change the compost-biochar interactions in mixtures and their amendment effects. Indeed, several studies observed an alleviation of beneficial effects of biochar-compost addition on biomass production over time [31–33]. However, to the best of our knowledge, no studies have focused on the effect of weathering on biochar-compost mixture properties and their biological stability.

Therefore, the aim of the present study was to investigate the effect of artificial weathering on chemical characteristics and biological stability of biochar-compost mixtures and the consequences for plant biomass production after soil amendment. We used two industrially produced biochars from maize and *Miscanthus*, a green-waste compost and the corresponding biochar-compost mixtures. The mixtures and pure media were subjected to a physical weathering to mimic natural aging mechanisms. Thanks to contrasting stable carbon isotope ratios of biochars derived from C4 plants and compost derived from C3 plants, we were able to monitor the mineralization of the two components of the mixtures during a 1-year of laboratory incubation with a soil inoculum. In addition, we investigated in a 4-weeks pot experiment the effect of fresh and weathered biochar-compost mixtures on ryegrass growth growing on two different soils. We hypothesized that (i) biochar addition to compost would induce synergetic effects on biological stability and plant growth and that (ii) physical weathering would weaken these interactions.

2. Materials and Methods

2.1. Biochar and Compost

Biochars were produced from maize cobs (*Zea mays* L.) and elephant grass (*Miscanthus* × *giganteus*, Greef and Deuter), through pyrolysis without oxygen during 10 min at respectively 450 and 550 °C. Pyrolysis was performed by VTGreen (Allier, France), using an industrial pyrolysis reactor (Biogreen®Pyrolysis Technology, ETIA, Oise, France). The

compost was made from green wastes at the platform of Fertivert (Normandy, France). The composting process consisted of 4 months fermentation and 2 months maturation. Three compost turnings were applied. The biochar from maize cobs and the compost are the same than the ones used in Nobile et al. [34]. General parameters of the biochars and the compost are listed in Tables 2 and 3. Biochar-compost mixtures were prepared by mixing 20 % (*w*/*w*) of each biochar with 80% (*w*/*w*) of the compost. The biochars and mixtures were air-dried at ambient temperature and the compost was stored at 4 °C.

2.2. Physical Weathering

The mixtures and pure media were subjected to a physical weathering through wet-drying and freeze-thawing cycles to mimic natural aging mechanisms. The weathering procedure was inspired by Naisse et al. [29]. Briefly, we placed 100 g (d.w.) of compost or biochar-compost mixtures in PVC cylinders (ø 9.5 cm). Two PVC cylinder (ø 5 cm) were used for the weathering of 30 g of maize and *Miscanthus* biochars. We covered the bottom of all tubes with a polyamide canvas with 20 µm mesh size (SEFAR-Nitex, Sefar AG, Haiden, Switzerland) and placed them on smaller tubes of 10 cm height to elevate the device. All was then put in a 10 cm ø beaker, in order to recover the lixiviates (Supplementary Material, Figure S1). We mimicked weathering processes through three successive cycles including three cycles of wetting/drying and three cycles of freezing/thawing. Wetting/drying steps consisted of saturating the samples with distilled water, leaving them at room temperature during 3 h followed by drying of the sample at 60 °C overnight. Freezing/thawing steps consisted of saturating samples with distilled water with the same amount as for the previous cycles, freezing at −20 °C overnight and thawing during 6–7 h at 28 °C. We replicated these experiments 2 times. At the end of the weathering procedure, we dried the solid samples at 60 °C during 2 days and lixiviates until complete evaporation. Mass and carbon loss after artificial weathering were assessed by mass balance.

2.3. Material Properties: Physico-chemical, Elemental and Thermogravimetric Analysis

To measure pH and electrical conductivity (EC), 2 g of sample were mixed with 40 mL of distilled water and centrifugated for 1 h. The pH (780 pH meter, Metrohm, Herisau, Switzerland) was measured in the supernatant and the mixtures were filtered (glass microfibres paper, Fisherbrand) before EC (InLab® 738-ISM, Mettler Toledo, Columbus, Ohio, USA) measurement. We evaluated the effect of weathering on dissolved organic carbon content (DOC) and elemental content. For DOC determination, 2 g of dried samples were sieved at 2 mm and mixed with 40 mL of distilled water, (1:20 w/v) ratio. The samples were shaken during 1 h, centrifugated at 4750 t/min during 20 min and the supernatant recovered by filtration (glass microfibres paper, Fisherbrand). DOC was analysed using a Total organic carbon analyzer (TOC-5050A, Shimadzu, Marne-la-Vallée, France). The determination of C, H, N and O of solid samples was performed using a CHN-O analyzer (FlashEA 1112 Series, Thermo-Fisher Scientific, Illkirch, France).

Ash content, volatile matter and fixed carbon of dry matter were determined by thermogravimetric analyses (TGA/DSC1 STAR System, Mettler-Toledo, Viroflay, France). The samples (in 70 µL crucibles, 3approx. 6–7 mg) were first heated at 105 °C during 30 min to determine the moisture content. Thereafter, the temperature was increased by 15 °C min^{-1} to 900 °C during 40 min under N_2 atmosphere to determine volatile content. Temperature was then kept at 900 °C under air flux (50 mL min^{-1}) for 6 min to determine ash content.

2.4. Biological Stability: Incubation

Laboratory incubation was carried out under optimum conditions after the addition of a microbial inoculum (4 mL soil inoculum per 100 g of sample). The inoculum was prepared with 50 g of soil from a cropland field (Haplic Luvisol [35], Beauvais, Northern France), by preparing a water extract with 200 mL of distilled water. The soil was not carbonated, contained 154 mg g^{-1} organic C, 18 mg g^{-1} total N and had a pH (water) of 7.7

(Table 1). After inoculum addition, 20 g of sample were placed in 100 mL glass vials and covered with rubber septa. We carried out the incubation in triplicate for 8 treatments (2 biochar/compost mixtures, a compost and one biochar (all fresh and weathered) at 20 °C during 12 months. As we hypothesized that pure biochars will behave similarly, we used only *Miscanthus* biochar as control sample. We adjusted the water content to 60 % at the beginning of the incubation, when the flask's atmosphere was free of CO_2. We monitored the decomposition of the materials by measuring release of CO_2-C using a micro-GC (490 Micro-GC, Agilent Technologies, Les Ulis, France) and the stable carbon isotope ratio of CO_2-C with an isotopic ratio mass spectrometer (Vario isotope select, Elementar, UK-Ltd, Cheadle, UK) at day 1, 3, 7, 16, 24, and then once a month until the end of the incubation. At each CO_2-C measurement date, we also determined the isotopic signature of the CO_2 emitted by compost, biochar and compost-biochar mixtures. Thanks to the isotopic ^{13}C signature of the C4-biochar, which is distinctly different from C3 compost, we were able to determine the contribution of carbon mineralized from biochar or compost in CO_2 emitted from the biochar-compost mixtures. After each measurement, we flushed the bottles with synthetic CO_2 free-air. The results are expressed as cumulated CO_2-C emitted form fresh and aged samples in terms of initial total C content of the compost or biochar within the fresh samples.

2.5. Effect on Biomass Production: Pot Experiment

A pot experiment was carried out with fresh and weathered compost and mixtures added to two different agricultural soils sampled in Beauvais (Northern France) and classified as a silt loam Haplic Luvisol and a clay loam Calcaric Cambisol [35]. Soil characteristics are shown in Table 1.

Table 1. Characteristics of the Calcaric Cambisol and Haplic Luvisol used for the pot experiment.

	Unit (Dry Matter)	Calcaric Cambisol	Haplic Luvisol
Clay	%	33.3	17.6
Silt	%	46.1	66.9
Sand	%	20.6	15.6
CaCO3	g kg^{-1}	563.3	0.0
organic C	g kg^{-1}	9.5	15.4
total N	g kg^{-1}	2.6	1.8
C/N		3.6	8.6
pH KCl		7.8	7.4
pH water		8.0	7.8
CEC	cmolc kg^{-1}	14.0	12.5
P water	mg kg^{-1}	1.2	3.9
Available P	mg kg^{-1}	19.7	71.2
Available K	mg kg^{-1}	326.8	291.9
Available Mg	mg kg^{-1}	271.1	100.7
Available Ca	mg kg^{-1}	46727.4	3868.6

After sieving the soil (4 mm), the composts and mixtures were applied at respectively 16t ha^{-1} and 20 t ha^{-1} to 0.4 kg of soil. Both fresh and weathered amendments were applied to soil at a similar rate, considering the mass loss during the weathering treatment. The pots were sown with 0.15 g pot^{-1} of Italian ryegrass (*Festuca perennis* Lam. ex *Lolium multiflorum*) seeds. Thereafter, they were kept in a growth chamber under controlled conditions: 16 h day^{-1} of light, a temperature of 24 °C (day) and 20 °C (night) and addition of distilled water every two days (Supplementary Material, Figure S2). We harvested the plants 4 weeks after sowing by cutting at 2 cm from soil surface. Biomass production was determined gravimetrically after 72 h drying at 60 °C.

2.6. Calculations and statistics

The stable C isotope signatures were used to estimate the contribution of biochar and compost to the mixtures and the CO_2 emissions from the mixtures. The partitioning was done with Equation (1):

$$C_{biochar,mix} = (\delta^{13}C_{mixture} - \delta^{13}C_{compost})/(\delta^{13}C_{biochar} - \delta^{13}C_{compost}) \quad (1)$$

where $C_{biochar,mix}$ is biochar carbon in the mixture or in CO_2-C emitted from the mixture (%); $\delta^{13}C_{mixture}$ is the stable C isotope signature of the mixture, $\delta^{13}C_{biochar}$ is the stable C isotope signature of biochar and $\delta^{13}C_{compost}$ is the stable isotope signature of compost.

To evaluate interactions between biochar and compost in mixtures, we calculated expected values for the mixtures according to Equation (2). The comparison between the expected and the measured values of the mixtures were used to assess interactions between biochar and compost.

$$m_{biochar,mix}/m_{mixture} = C_{mixture} \times C_{biochar,mix}/C_{biochar} \quad (2)$$

where $m_{biochar,mix}$ is the mass of biochar within the mixture (g); $m_{mixture}$ is the mass of the mixture (g); $C_{mixture}$ is the C content of the mixture; and $C_{biochar}$ is the C content of biochar.

To calculate differences between fresh and weathered materials, we tested for normality using the Shapiro-Wilk test. For the normally distributed data, we performed analysis of variances (ANOVA) and Tukey multiple comparison. When data did not follow a normal distribution, we used Kruskal-Wallis tests with Bonferroni corrections. The level of significance was set at $p = 0.05$. We performed all statistical analyses using the R software (version 3.5.2).

3. Results

3.1. Leaching Due to Physical Weathering

Material losses ranged from about 20 mg g^{-1} for maize biochar to about 150 mg g^{-1} for compost (Figure 1). Artificial physical weathering thus resulted in twice as much material loss from compost as compared to biochars. Mass losses for both mixtures were around 75 mg g^{-1}. They were about two times lower than expected from the losses of individual materials (Figure 1).

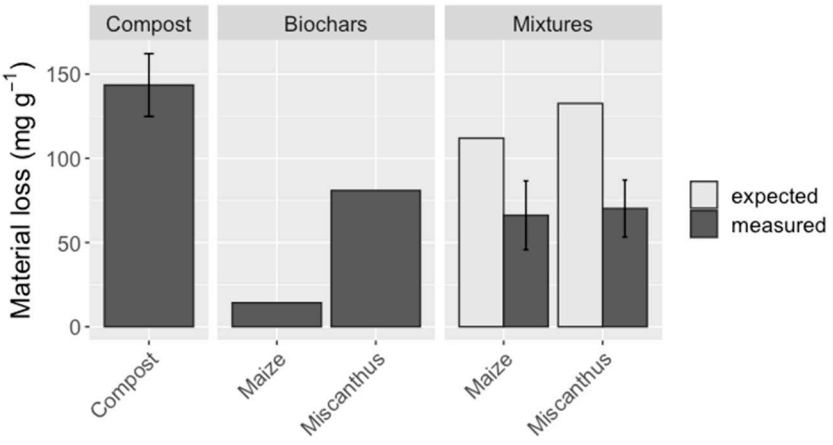

Figure 1. Total mass loss during physical weathering of compost, biochars and their mixtures. Data are presented as mean ± sd ($n = 2$ for the compost and the mixture and $n = 1$ for the biochars). Expected values for mixtures were calculated based on mass losses measured for individual components.

3.2. Properties of the Fresh and Weathered Materials

3.2.1. Elemental Composition

Fresh compost was composed of 226 mg g^{-1} C, 20 mg g^{-1} H, 112 mg g^{-1} O and 23 mg g^{-1} N (Table 2). Fresh biochars contained at least twice more C than the fresh compost, with biochar from maize and *Miscanthus* containing respectively 591 and 778 mg g^{-1} C (Table 2). Hydrogen content of biochars were similar to compost, whereas O and N content of biochars were at least twice lower than for compost. Following the mixing ratio, carbon content of the mixtures ranged between 298 mg g^{-1} and 332 mg g^{-1} and all other elemental components had similar values for both mixtures. The mixtures showed similar C/N ratios independently from biochar feedstocks.

Table 2. Elemental composition of fresh (F) and weathered (W) compost, biochars and biochar-compost mixtures. Expected (exp) values were calculated for the weathered mixtures. Data are presented as means ± sd (n = 3). The letters represent differences among treatments.

			C (mg g^{-1})	H (mg g^{-1})	O (mg g^{-1})	N (mg g^{-1})	C/N
Compost							
Compost	F		226 ± 1 ^{i}	20 ± 1 ^{ab}	112 ± 4 ^{a}	23 ± 0 ^{a}	10 ± 0 ^{g}
	W		209 ± 5 ^{j}	17 ± 5 ^{abc}	99 ± 4 ^{b}	21 ± 1 ^{ab}	10 ± 0 ^{g}
Biochars							
Maize	F		591 ± 1 ^{d}	21 ± 1 ^{ab}	48 ± 3 ^{d}	8 ± 0 ^{gh}	72 ± 0 ^{c}
	W		618 ± 0 ^{c}	21 ± 0 ^{ab}	76 ± 5 ^{c}	9 ± 0 ^{fg}	65 ± 0 ^{d}
Miscanthus	F		778 ± 1 ^{a}	13 ± 1 ^{c}	18 ± 3 ^{e}	4 ± 0 ^{hi}	186 ± 0 ^{b}
	W		742 ± 1 ^{b}	16 ± 2 ^{bc}	58 ± 6 ^{d}	4 ± 0 ^{i}	189 ± 0.3 ^{a}
Mixtures							
Maize	F		298 ± 3 ^{h}	19 ± 1 ^{ab}	103 ± 0 ^{ab}	17 ± 0 ^{ef}	17 ± 0 ^{f}
	W		350 ± 3 ^{f}	22 ± 1 ^{a}	78 ± 1 ^{c}	18 ± 0 ^{cd}	20 ± 0 ^{ef}
		exp	321	18	92	18	18
Miscanthus	F		332 ± 1 ^{g}	19 ± 2 ^{ab}	107 ± 3 ^{ab}	19 ± 0 ^{bc}	17 ± 0 ^{f}
	W		374 ± 3 ^{e}	20 ± 1 ^{ab}	83 ± 1 ^{c}	17 ± 0 ^{de}	22 ± 0 ^{e}
		exp	355	16	87	16	22

Compost weathering induced decreasing contents of all elements, while mostly C and O were affected for biochars. As a result of weathering, C content respectively increased and decreased for the maize and *Miscanthus* biochars, while O content more than doubled for both biochars. The expected C content of the weathered mixtures were slightly lower than the measured ones ranging between 321 and 355. As for biochars, weathering affected mainly the C and O contents of the mixtures; O contents of the weathered mixtures were slightly lower than the expected values. For both mixtures, weathering increased the C/N ratio (Table 2).

3.2.2. Physico-Chemical Properties, Dissolved Organic Carbon and Stable $\delta^{13}C$ Ratio

Table 3 shows physico-chemical properties and the dissolved organic carbon content (DOC) of the materials. pH and electrical conductivity (EC) ranged from 8.1 to 10.5 and from 109 to 1598 μS cm^{-1}, respectively. Compost had lower pH (8.4), and EC (944 μS cm^{-1}) than both biochars. Both biochars showed similar pH (around 10.5), but maize biochar had higher EC than *Miscanthus* biochar. The pH and EC of fresh mixtures were in between the values from compost and biochars.

Fixed C content ranged between 0.6 and 67.8 %, DOC varied between 2.2 and 277.2 mg g^{-1} C, whereas ash content ranged between 13.6 and 59.3 % and volatile matter content between 17.8 and 38.8 %. Compost showed lower fixed C and higher DOC, ash content and volatile matter than biochars. Both biochars had similar volatile C but varied in ash content and fixed C; maize biochar presented a twice-higher ash content and a lower fixed C content (45.6 vs. 63.6%) than *Miscanthus* biochar. We assumed that differences between

the two biochars were mainly driven by production temperature rather than initial feedstock, as it has been found to be the main driver of biochar chemical composition [36–38]. Maize mixtures showed higher pH (9.1 vs. 8.9) and ash contents (54.0 vs. 51.2%) and lower volatile matter contents (35.1 vs. 38.2%) compared to *Miscanthus* mixture.

Table 3. Chemical characteristics of fresh (F) and weathered (W) compost, biochars and biochar-compost mixtures. Expected (exp.) values were calculated for the weathered mixtures. EC: electric conductivity; DOC: dissolved organic carbon. Data are presented as means ± sd (n = 3) for pH, EC, DOC and $\delta^{13}C$. Proximate analysis was carried out for 1 sample. The letters represent differences among treatments.

			pH *	EC ($\mu S\ cm^{-1}$)	DOC ($mg\ g^{-1}$ C)	$\delta^{13}C$ (‰)	Ash (%)	Volatile (%)	Fixed C (%)
Compost									
Compost	F		8.4 ^{g}	944 ± 18 ^{cd}	277.2 ± 49.0 ^{a}	−28.9 ± 0.1 ^{gh}	59.3	38.8	1.9
	W		7.9 ^{h}	215 ± 4 ^{fg}	73.5 ± 2.4 ^{cd}	−29.2 ± 0.0 ^{h}	63.0	36.4	0.6
Biochars									
Maize	F		10.5 ^{a}	1640 ± 62 ^{a}	36.7 ± 1.6 ^{f}	−15.3 ± 0.1 ^{bc}	28.5	25.9	45.6
	W		8.7 ^{e}	109 ± 3 ^{g}	15.5 ± 0.1 ^{fg}	−15.3 ± 0.0 ^{c}	23.5	35.7	40.8
Miscanthus	F		10.4 ^{a}	1516 ± 14 ^{bc}	3.6 ± 0.7 ^{g}	−14.9 ± 0.1 ^{ab}	13.6	22.8	63.6
	W		9.4 ^{b}	129 ± 3 ^{g}	2.2 ± 0.1 ^{g}	−14.5 ± 0.1 ^{a}	14.4	17.8	67.8
Mixtures									
Maize	F		9.1 ^{c}	1588 ± 12 ^{ab}	203.2 ± 7.9 ^{bc}	−22.3 ± 0.3 ^{ef}	54.0	35.1	10.9
	W	real	8.6 ^{e}	224 ± 3 ^{ef}	50.1 ± 1.1 ^{ef}	−21.9 ± 0.0 ^{de}	48.9	34.0	17.1
		exp	8.1	186	57.6	−25.4	52.2	36.2	11.6
Miscanthus	F		8.9 ^{d}	1598 ± 20 ^{a}	210.3 ± 9.3 ^{ab}	−23.2 ± 0.1 ^{fg}	51.2	38.2	10.6
	W	real	8.5 ^{f}	238 ± 15 ^{de}	54.3 ± 1.5 ^{de}	−21.9 ± 0.1 ^{d}	46.3	37.1	16.6
		exp	8.3	192	54.0	−25.2	49.7	31.3	19.0

* standard deviations of pH were <0.05.

Weathering induced an increase of fixed C from around 10% to 17.1% and 16.6% for maize and *Miscanthus* mixtures. In contrast, EC and DOC showed 4 times lower values after weathering. When compared to the expected values, slightly higher EC values than expected were recorded for both mixtures after weathering. In addition, the weathered mixture with maize biochar showed lower DOC (50.1 vs. 57.6 mg g C^{-1}) and higher fixed C (17.1 vs. 11.6%) than expected. The weathered *Miscanthus* mixture showed higher volatile matter than expected (37.1 vs. 31.3%) (Table 3). During weathering, the isotopic signatures remained unchanged for compost, biochars and the mixture containing maize biochar, but decreased for the mixture containing *Miscanthus* biochar. The $\delta^{13}C$ ratios of the weathered mixtures (21.9‰) were lower than expected (25.4 and 25.2‰).

3.3. Biological Stability

Cumulative CO_2-C released during 1-year of incubation from fresh and weathered compost, *Miscanthus* biochar and both mixtures are presented in Figure 2. After 1 year of incubation, the fresh compost showed the highest cumulative C mineralization with values up to 30 mg g^{-1} of initial carbon. In contrast, very few C was mineralized from*Miscanthus* biochar. The isotopic signatures of carbon were used to assess the origin of C mineralized from biochar-compost mixtures. The data indicated that compost released between 15 and 20 mg g^{-1} C when incubated in mixtures, while biochar released between 10 and 15 mg g^{-1} C when incubated in mixtures. Compost showed lower C-mineralization in mixture compared to individual incubation. Conversely, biochar showed higher C-mineralization when combined with compost compared to individual incubation.

After weathering, cumulative compost C mineralization amounted to 10 mg g^{-1} C, which was significantly lower than C mineralization of fresh compost (Figure 2). Biochar C-mineralization was not significantly affected by weathering when individually incu-

bated. When combined with compost it mineralized significantly less than in fresh mixtures. In contrast, compost mineralized significantly more in weathered mixtures as compared to fresh mixtures and reached values between 20 and 25 mg g^{-1} C after 1-year incubation.

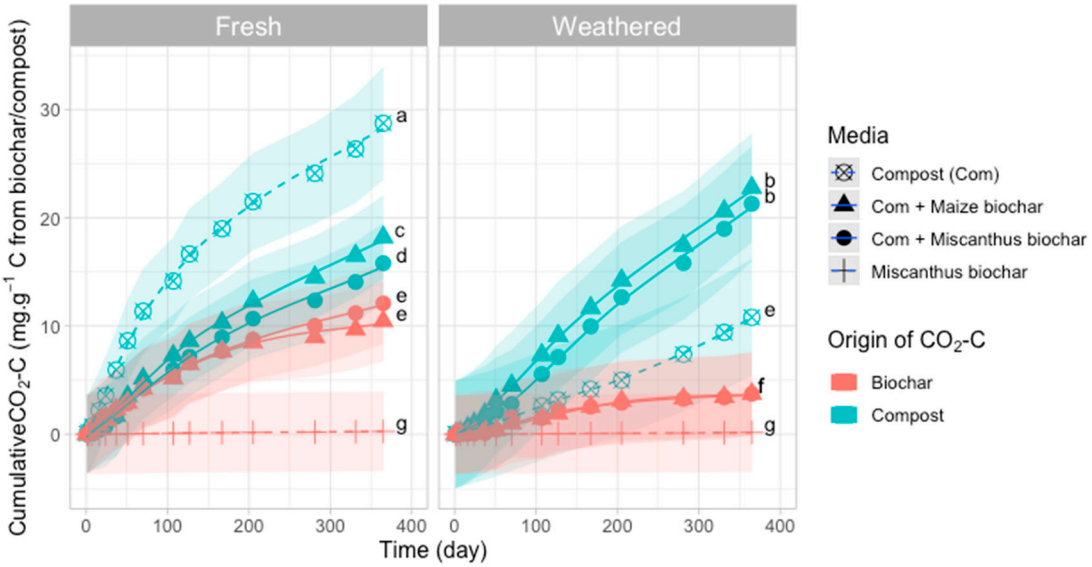

Figure 2. Cumulative CO_2-C mineralized from biochar and compost when incubated alone or in mixture. Turquoise and red colors represent C mineralized from compost and biochar respectively. Data represent means from 3 replicated samples. The colored ribbon represents the standard deviations. The letters represent the significant differences from a two-ways ANOVA analysis ($n = 3$).

3.4. Ryegrass Growth

Biomass of Italian ryegrass was higher when grown on Haplic Luvisol as compared to Calcaric Cambisol, as shown for the unamended controls (Figure 3). All organic amendments stimulated ryegrass growth, when applied to Calcaric Cambisol. However, when applied to Haplic Luvisol, organic amendments induced neutral or negative effects on biomass. For both soils, application of fresh biochar-compost mixtures did not lead to significant differences in ryegrass biomass as compared to fresh compost alone. Physical weathering decreased the effect of compost addition to Calcaric Cambisol on biomass, but the effect was still positive as compared to the control. Concerning the Haplic Luvisol, compost addition tended to decrease biomass. For both soils and after weathering, the mixture containing *Miscanthus* biochar induced significantly higher biomass than the compost alone, while the mixture containing maize biochar showed similar effects as compost alone.

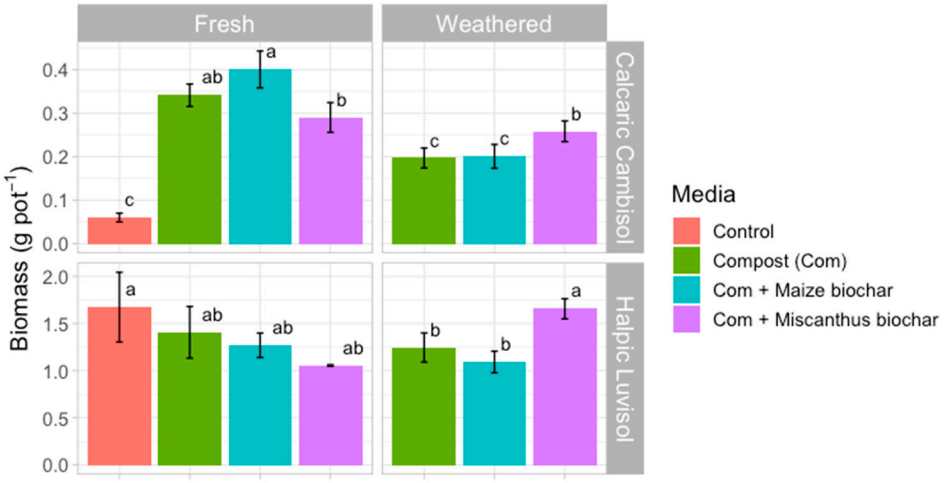

Figure 3. Biomass of ryegrass after addition of compost or its mixture with maize and *Miscanthus* biochars, grown on two soil types. Data are presented as means ± sd (n = 3). The letters represent the significant differences from a one-way ANOVA analysis (n = 4) within each treatment and soil type.

4. Discussion

4.1. Weathering Effects on Material Properties

Physical weathering induced much higher mass loss from compost as compared to biochar and mixtures. This may probably be explained by the high leaching losses. Biochar mass loss amounted to 75 mg g^{-1}, which is much lower than observed for gasification biochar [29]. This may be due to the lower friability of biochar produced by pyrolysis making it less prone to particle losses [30]. Lower mass loss for the mixtures than expected (Figure 1), may be explained by protection of compost from leaching losses by its association with the biochar structure [12,39]. Both weathering cycles may affect release of dissolved organic matter and cause cracking on biochar-surfaces, thus leading to changes in pore structure [40]. While DOC was lower than expected in weathered mixtures, EC values were higher than expected (see below). We therefore suggest that there may be interactions between biochar and compost leading to solid particles retention during weathering treatment.

Compost weathering induced a decrease of the content of all main elements, following strong leaching due to weathering treatment (Table 2). However, weathering of biochars affected only C and O contents and led to decreasing C content and increasing O content. Our results are consistent with data of Naisse et al. [29], who suggested that these observations may indicate oxidation processes induced by weathering [41]. In contrast, weathering of the mixtures increased their C contents, while it decreased their O contents. This might be related to a preferential elimination of O relative to C in the labile fraction of the mixtures. This hypothesis may be supported by the visual observation of high loss of soluble compounds during weathering. Indeed, strong decreases of DOC and EC of the remaining substrates indicated that soluble compounds were removed by leaching during artificial weathering (Table 3). In contrast to the mixture containing *Miscanthus* biochar, the DOC content of the mixture containing maize biochar decreased slightly stronger than expected. The strong decrease of EC as a result of weathering is consistent with the results of Yao et al. [42], who evidenced a rapid decline of EC from 0.7 to 0.2 mS cm^{-1} following leaching losses from biochar. EC reduction after weathering may be due to the leaching of mineral biochar compounds. This is supported by the lower ash content of the material remaining after weathering. Ashes and volatile compounds were both partly removed during weathering, except for volatile compounds of maize biochar. Both ashes and volatile compounds compose the labile fraction of all materials and are more likely to be

leached than the more stable compounds. In particular, ash represents the mineral material contribution, which may be an indicator of nutrient content [43].

Fixed C slightly decreased for compost and biochars following weathering treatment, while it increased for the mixtures (Table 3). Fixed C is mainly composed by fused aromatic C structures and may be used as an indicator of the C sequestration potential of biochars [44]. Higher fixed C of the mixtures than the expected values after weathering might result from the increasing chemical recalcitrance of the materials due to labile compounds leaching. These observations are in agreement with the lower than expected $\delta^{13}C$ ratios of the mixtures, might indicate preferential leaching of ^{12}C enriched compounds, e.g., C3-compost or labile polysaccharides, which are ^{13}C enriched compared to recalcitrant compounds [45].

4.2. Biological Stability

4.2.1. Biological Stability of the Fresh Materials

During the incubation, compost showed the highest cumulative C-mineralization, while biochar C hardly mineralized. C-mineralization of the mixtures ranged between those of its individual components. These results are in agreement with other studies [13,14,16] and may be explained by a higher content of labile C in compost than in biochar [5]. It was interesting to note that compost showed a lower C-mineralization when combined with biochar than when incubated individually. Two mechanisms could explain observation: the adsorption of labile fraction on the biochar surface [13], and the presence of phenolic compounds or salts originating from biochar [24,25,27], which might inhibit microbial activity in compost-biochar mixtures. The opposite effect was observed for biochar, since biochar C mineralized more when combined with compost than when individually applied. Indeed, several studies showed positive priming effect when labile substrates were added to biochar [46–48].

4.2.2. Effect of Weathering on the Biological Stability

The cumulative C-mineralization from compost after 1 year of incubation was significantly lower for weathered compost compared to fresh compost when individually incubated (10 vs. 30 mg g^{-1}). This negative effect of weathering on C-mineralization from compost was attributed to the strong leaching of easily mineralizable labile components. On the other hand, the absence of weathering effects on biochar C mineralisation may be explained by the high stability of biochar with only few labile compounds [48].

C-mineralization from compost in the mixture increased significantly after weathering, when compared to the fresh mixtures (Figure 2). This may be due to the protection of labile compounds by biochar and/or the removal of biochar compounds, which inhibited microbial activity and thus C-mineralization from compost (see above). Indeed, fresh biochar may contain large amounts of salts, which may inhibit microbial activity when applied to soil [49–51]. This could lead to the negative priming effect of biochar on native C often observed immediately after soil addition [52].

Weathering also reduced biochar C-mineralization, within the mixtures (Figure 2), most probably due to the leaching of easily mineralizable C and nutrients from compost, which stimulated biochar C-mineralization before weathering (see above). Our results thus indicate that weathering affects biochar-compost interaction in mixtures, which might also impact their effects on plant growth.

4.3. Ryegrass Growth

4.3.1. Effect of the Fresh Media on Ryegrass Growth

Higher ryegrass biomass was recorded when grown on Haplic Luvisol as compared to Calcaric Cambisol, regardless the organic amendment (Figure 3). Moreover, the addition of organic amendments containing compost had positive effects on biomass when applied on Calcaric Cambisol, but the effects were neutral or negative when applied to

Haplic Luvisol (Figure 3). Our results were consistent with the results of Von Glisczynski et al. [53], who also did not find any plant growth promoting effect of biochar-compost mixtures application on Haplic Luvisol. As reviewed by Faucon et al. [54], organic amendments such as compost may promote plant growth by providing readily available nutrients or releasing them through mineralization. The available P concentration of the Calcaric Cambisol was much lower than that of the Haplic Luvisol (19.66 vs. 71.18 mg kg^{-1}) (Table 1), suggesting a possible P-limitation for plant growth in this soil, which might have been alleviated by compost application.

Addition of biochar compost mixtures led to similar ryegrass biomass than compost along (Figure 3). As reported in the literature, the combination of biochar with compost can have synergic [32,55], antagonistic [23,56] or neutral effects [16,18,23,57,58] on plant growth. Several factors may impact plant growth after biochar-compost mixtures addition and the mechanisms are still poorly understood [17]. It was suggested that pre-treatment of biochar may be beneficial for plant growth before its soil application [59]. Moreover, it was shown that weathering may alter biochar properties [29]. Therefore, we tested in the following, if weathering of biochar/compost mixtures influenced plant growth.

4.3.2. Effect of Weathered Amendments on Ryegrass Growth

Irrespective of the soil type, weathered compost had negative or neutral effects on biomass when individually applied (Figure 3). This is most likely due to the weathering-induced loss of readily-available nutrients and easily-mineralizable C compounds (Table 3 and Figure 2).

The addition of weathered biochar-compost mixtures to both soils had neutral or positive effects on biomass compared to the effect of compost applied individually depending on the biochar feedstock (Figure 3). The positive effect of the weathered *Miscantus* mixture on biomass may result from better compost mineralisation through the removal of compounds, which inhibit microbial activity as discussed above (Section 4.3.1). However, the weathered maize mixture showed neutral effect on biomass when compared to the effects of weathered compost alone. Our results showed that weathering of biochar-compost mixtures could lead to positive growth effect. These results are in agreement with a recent field study, showing positive growth effects on the second crop after soil application [60]. In addition, our results also showed that neutral effects of weathering depending on biochar feedstocks and/or soil type may occur [60,61]. Further studies would be needed to investigate the mechanisms controlling the variation of biochar-compost interactions on plant growth over time.

5. Conclusions

We investigated the effect of two biochar-compost mixtures and weathering on their material properties, biological stability and on plant growth after addition to two contrasting soils. Our results showed that the physical weathering led to the alteration of material properties of the mixtures, in particular through leaching of labile compounds. These effects could impact the mineralisation of the mixture and also plant growth after soil addition. We suggest that the mixtures contained inhibitive compounds for microbial activity in their labile fraction, as shown by the negative effect on compost mineralisation when combined with biochar. The increase of compost mineralisation within the mixtures after weathering may have provided more plant available nutrients, which could promote plant biomass production when compared to individual compost application. On the other hand, biochar mineralisation was also affected by weathering, indicating that weathering may influence its C sequestration potential.

We conclude that biochar-compost interactions are evolving after physical weathering most probably due to its effect on leaching of soluble compounds. The effect of fresh and weathered biochar-compost mixtures on plant growth depend on biochar production conditions. Further studies should focus on mechanisms influencing the nutrient supply of biochar-compost mixtures.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4395/11/2/336/s1, Figure S1: Experimental set up used for physical weathering of organic amendments, Figure S2: Pot experiment with ryegrass.

Author Contributions: M.-L.A., C.G., S.H., C.N., D.H. and C.R. designed the study. M.-L.A. and C.N. carried out the laboratory work, and exploited the results. Y.L.B. and S.B. contributed data. M.-L.A. wrote the first draft of the manuscript. All authors discussed the results and commented on the manuscript.

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