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Biomass Ash as a Substitute for Lime and Its Impact on Grassland Soil, Forage, and Soil Microbiota

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Abstract: In this eight-year grassland field trial, we compared the fertilization effects of biomass ashes (BMAs) and carbonated lime ($CaCO_3$) in combined application with cattle slurry (CS). Our study focused on plant coverage, forage yield, and quality, as well as soil physicochemical and microbiological properties. The fertilization strategies included CS mixed with BMA or $CaCO_3$ applied three times a year and a separate annual application of ash or $CaCO_3$, independent of CS. Samplings were performed in 2010, 2014, and 2018. Despite an absence of observable effects on soil, microbial properties, and forage quality, CS application, with or without BMA/$CaCO_3$, resulted in higher forage yields compared to the unfertilized control and plots receiving only ash or $CaCO_3$. Forage properties remained consistent across treatments. However, the combined application of CS with both ash and $CaCO_3$ led to a reduction in volatile organic compounds, total carbon, total nitrogen, nitrate, and electrical conductivity in the soil from 2010 to 2018. Additionally, the relative abundance of specific microbial families (Nitrosomonadaceae, Acidothermaceae, Bacillaceae, and Peptostreptococcaceae) varied based on whether soils received a single amendment or a combination thereof. Our findings suggest that BMA is a valuable substitute for traditional liming agents, regardless of the application mode.

Keywords: liming; biomass ash; forage growth; microbial properties; long-term field trial; recycling

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1. Introduction

The use of renewable energy is being promoted as a cost-efficient and environmentally friendly alternative to fossil fuels [1]. In 2018, 21.1% of the total energy used for cooling or heating came from renewable sources; this makes an increase of 11.7% when compared to 2004 [2]. In this context, the EU-28 energy policies are impelling the share of 27% renewable energies on the total energy consumption by 2030 [3]. Furthermore, recent conflicts and crises, like the COVID-19 pandemic and the invasion of Ukraine, showed the need for sustainable and easily available fertilizers, to secure food supply [4,5].

The utilization of biomass for energy production results in an accompanying rise in the generation of BMA as a by-product [6]. For instance, an amount of 202,000 tons of ash from untreated biomass (wood chips, bark, straw, and agricultural residues) was produced in Austria in 2020, a doubling since 2004 [7]. BMAs have an enormous potential

as a soil amendment or as a supplement to fertilizers in agriculture [8–10], as they contain macronutrients such as calcium (Ca), magnesium (Mg), potassium (K), and phosphorus (P), and micronutrients including iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu). Their deficit in carbon (C) and nitrogen (N), which are mostly volatilized during the combustion of the biomass, can be alleviated by combining the ashes with organic materials including compost or farm manure [11–13]. The buffering capacity of BMAs may also help to reduce solubility and availability of contained toxic heavy metals (HMs) and might, therefore, overcome potentially negative effects of the ashes on soils and plants [14,15]. Nevertheless, monitoring of HMs is advised to determine both the fertilization rate and regime that is safe to apply.

The use of biomass ashes as a substitute for traditional liming agents contributes to a circular economy and at the same time, it prevents their landfilling [16,17]. Despite previous studies having highlighted that BMAs may be used as a replacement of lime (e.g., quicklime, $CaCO_3$; or carbonated lime, $CaCO_3$) [17,18], others have found negligible or negative effects in the case of soil N-limitation [19]. This calls for the necessity of balancing ash amendments with N fertilization. Therefore, the application of ashes along with N-rich organic fertilizers, such as cattle slurry (CS), may result in additional benefits, particularly for acidic soils, as their nutrient contents complement each other [20,21]. According to Bougnom et al. [20], the use of CS leads to an increase in the forage yield, and a combination with wood ash (3 t ha^{-1}) further increased the yield. Similar results were found by Fernández-Delgado Juárez et al. [21]. Paz-Ferreiro et al. [22] applied both wood ash and lime to mixed mountain pastures and compared their effects. They found that only the concomitant application of wood ash with an external source of P and K resulted in an increase in forage yield.

The addition of BMA may affect not only the soil chemistry and forage yield but also microbiological variables, such as soil microbial biomass, activity, and community composition [9,11,23–25]. Microbial properties are sensitive indicators when evaluating soil disturbances, as they respond sensibly to changes in soil management [26]. For instance, Zimmermann and Frey [27] found a rapid increase in soil microbial biomass, followed by a decrease two months after the application of 8 t ha^{-1} of wood ash to a forest soil. Perucci et al. [28] found that the addition of 20 t ha^{-1} ash on an agricultural system had a pronounced effect on soil physicochemical and microbiological properties, but the effects disappeared 12 months after the treatment. In terms of community composition, Bang-Andreasen et al. [25] showed that BMA amendment of 3 and 12 t ha^{-1} decreased the abundance of the copiotrophic groups *Chitinonophagaceae* (Bacteroidetes) and *Rhizobiales* (Alphaproteobacteria) in an agricultural soil.

Many studies have focused on short-term effects [10,21,29] and some were confined to a laboratory scale [25,30,31]. While a few of these studies explored properties akin to our investigation [10,21] or involved long-term trials [32], none comprehensively considered the diverse range of variables fundamental to our study. Here, we aimed at closing this knowledge gap by comparing the effects of BMAs and the liming agent $CaCO_3$ on soil and forage properties of a grassland system in an eight-year field trial. We studied both amendments either alone or in combination with cattle slurry. In addition, we tested two application strategies: (i) cattle slurry was mixed with BMA/$CaCO_3$ prior to their combined application onto the soil three times a year; (ii) BMA/$CaCO_3$ were applied once a year and separately from cattle slurry applied three times a year. We measured the soil basal respiration rate as a proxy for microbial activity, microbial biomass carbon (C_{mic}), and the metabolic quotient (qCO_2) prior to the start of the experiment in 2010 and later in 2014 and 2018, respectively. The composition and diversity of soil bacteria and fungi were determined at the end of the experiment in 2018. Ultimately, forage yield, forage quality, and botanical composition were analyzed in both 2014 and 2018.

We hypothesize that (i) BMA may serve as a substitute for traditional lime enhancing forage yield with and without the use of cattle slurry; (ii) BMA affects soil microbial and physicochemical properties enhancing microbial biomass and diversity and increases nutrient levels; (iii) the effects of BMA/lime are independent of the mode of application,

which is together with the slurry, or applied separately; the application of BMA does not result in HM accumulation, neither in soil (iv) nor forage (v).

2. Material and Methods

2.1. Field Trial and Sampling Strategy

The field trial was conducted at the Agricultural Research and Education Centre (AREC) Raumberg-Gumpenstein (Irdning, Austria) on a permanent meadow (47°29′36″ N 14°06′12″ E). For the years 2010, 2014, and 2018, the mean annual precipitation and temperature were 785 mm and 7.7 °C, 767 mm and 9.7 °C, and 738 mm and 9.6 °C, respectively. According to the WRB system [33], the soil was classified as dystric cambisol (arenic, humic). In 2007, the experimental field was ploughed and sown with a seed mixture, 'Dauerwiese-B' (B4), from the Austrian federation of grassland and ley-farming (ÖAG), which consisted of perennial ryegrass (*Lolium perenne* L.), tall oat grass (*Arrhenatherum elatius* L.), golden oat grass (*Trisetum flavescens* L.), orchard grass (*Dactylis glomerata* L.), red fescue (*Festuca rubra* L.), timothy (*Phleum pratense* L.), meadow foxtail (*Alopecurus pratensis* L.), Kentucky blue grass (*Poa pratensis* L.), meadow fescue (*Festuca pratensis* L.), birdsfoot trefoil (*Lotus corniculatus* L.), and white clover (*Trifolium repens* L.).

The bottom wood ash used in the present trial was obtained from a combined heat and power plant in Stainach (Styria, Austria), where bark, sawdust, and wood chips were used as input materials. The ash fulfilled the requirements established in the Austrian Guidelines for the use of BMA in forestry and agriculture [34]. The CS was provided by the stables for cattle breeding research of the Agricultural Research and Education Centre Raumberg-Gumpenstein (Irdning, Austria). The BMA and CS properties are listed in Table S1.

Organic fertilizer (i.e., CS) dosages were chosen according to the Austrian Fertilization Guidelines [35]. Ash was applied at a rate of 500 kg ha^{-1} $year^{-1}$, which is the legal maximum dosage applicable to grasslands according to the Austrian Guidelines for the use of BMA in forestry and agriculture [34]. Plots receiving solely $CaCO_3$ were treated with 56 kg ha^{-1} $year^{-1}$, i.e., matching the Ca amount applied to plots treated with BMA. For plots receiving only CS, it was applied at a rate of 90 kg N_{tot} ha^{-1} $year^{-1}$ three times each year (3 × 30 kg N_{tot} ha^{-1}), at the beginning of the growing season, and after the first and second cutting, respectively. The plots receiving one application of either $CaCO_3$ or BMA and CS but without mixing them previously received CS three times equal to 30 kg N_{tot} ha^{-1} and 56 kg ha^{-1} $year^{-1}$ $CaCO_3$ or 500 kg ha^{-1} $year^{-1}$ ash (167 + 167 + 166 kg ha^{-1} $year^{-1}$), respectively. The plots fertilized with a mixture of either $CaCO_3$ or BMA and CS received 90 kg N_{tot} ha^{-1} $year^{-1}$ of CS mixed with either 56 kg ha^{-1} $year^{-1}$ $CaCO_3$ or 500 kg ha^{-1} $year^{-1}$ ash in the autumn of each year.

The field trial ran from November 2010 to October 2018. A total of eight different fertilization treatments were set up with four replicate plots per treatment following a randomized block design: ***C*** = control (unfertilized); ***Lime*** = $CaCO_3$; ***BMA*** = biomass ash; ***CS*** = CS; ***CS + BMA*** = CS + biomass ash, mixed (3 applications); ***CS + Lime*** = CS + $CaCO_3$, mixed (3 applications); ***CSLime*** = CS + $CaCO_3$ (autumn application); ***CSBMA*** = CS + biomass ash (autumn application). For *CSLime* and *CSBMA*, the BMAs and $CaCO_3$ were applied in autumn (October–November) in order to avoid plant disturbances during the growing period. However, for the mixed treatments *CS + Lime* and *CS + Ash*, the BMA and $CaCO_3$ were mixed with CS and applied three times per year. The slurry was mixed with BMA or carbonated lime in the appropriate ratio in wide-necked drums and homogenized by stirring shortly before spreading. The precisely determined quantities were distributed manually on the plots.

Soil sample collection and soil analyses were performed before onset or at the end of the growing seasons on 22 April 2010, 6 October 2014, and 16 October 2018. For each plot (12.7 m^2), a composite soil sample consisting of 20 random subsamples from the top 10 cm layer was collected with an auger (3 cm diameter). All soil samples were gently mixed, sieved (fraction ≤ 2 mm), and stored at 4 °C or frozen (−20 °C) until further analyses.

2.2. Plant Cover and Yield

Total plant cover and species group distribution (proportion of grasses, legumes, and herbs) were determined visually according to Peratoner and Pötsch [36] three times a year shortly before harvesting. Total plant (forage) yield was measured right after each clipping three times a year, determining both fresh and dry matter yield. The side-strips (30 cm) of the plots were not sampled, thus avoiding possible edge effects.

2.3. Forage Analysis

Forage analyses were performed for all of the three clippings in 2014 and 2018. For each fertilization treatment, the forage samples from the four experimental plots were pooled. Samples were dried for 48 h at 45 °C in a ventilated oven. The dry matter content was determined by weighing, and the samples were grounded (Ø $\leq$ 500 µm) and stored at room temperature until a further analysis.

Crude fiber (CFR) was determined with a VELP Scientifica Fiber Analyzer (Usmate, Italy) [37] and crude fat (CFA) content was measured according to Weender by Soxhlet extration [38]. Crude protein content (CP) was assessed following the Dumas method [39] on a C/N/S Variomax (Elementar Analysensysteme, Langensebold, Germany). Ash content was determined after overnight ignition in an AHT muffle furnace (Nabertherm, Lilienthal, Germany) at 550 °C [40]. The concentration of Ca, Mg, K, and P was measured with atomic absorption spectrometry after the digestion of the ash with 3 M HCl. The contents of Na, Zn, Mn, Cu, and Fe were measured with atomic absorption spectrometry after digestion with 15% HCl. These analyses were performed with an icap 6300 duo ICP (Thermo Scientific, Vienna, Austria) [41].

2.4. Soil/Biomass Ash/CS Physicochemical and Microbiological Measurements

Soil samples (10 g fresh weight) were oven-dried (105 °C) for 24 h, and re-weighed (dry matter, DM). The maximum soil water holding capacity (WHC) was measured according to Öhlinger [42]. The actual soil water content was calculated as the percentage of the maximum WHC. The volatile organic compounds (VOCs) were determined from the weight loss of oven-dried soil following ignition in a muffle furnace (Carbolite CWF 1000, Carbolite Gero, Neuhausen, Germany) at 550 °C for 5 h. Total C and N were analyzed from dried samples, using a TruSpec CHN analyser (LECO, St. Joseph, MI, U.S.A.). Electrical conductivity (EC) and pH were measured in distilled water and 0.01 M $CaCl_2$ extracts (10:25, *w*/*v*), by using conductivity meter LF 330 (WTW, Weilheim, Germany) and a pH meter (Metrohm 744, Metrohm, Herisau, Switzerland), respectively. Ammonium (NH_4) and nitrate–nitrogen (N-NO_3) concentrations were analyzed according to Kandeler [43,44]. To determine the potential nitrogen mineralization (PNM), soil samples were saturated with water for 7 d, and the ammonium released was measured according to Kandeler [45]. Potential nitrification (Nit) was measured following the method by Kandeler [46]. Basal respiration (BR) and microbial biomass (C_{mic}) were measured according to Heinemeyer et al. [47]. The metabolic quotient (qCO_2, µg CO_2–C g^{-1} C_{mic} h^{-1}) was calculated from BR and C_{mic} according to Anderson and Domsch [48]. Soil cation exchange capacities of different elements (Ca_{ex}, Mg_{ex}, K_{ex}, Na_{ex}, Al_{ex}, Fe_{ex}, Mn_{ex}) were analyzed according to ÖNORM L 1086-1 [49] (barium chloride solution) and total HM contents according to ÖNORM L 1085 [50] (digestion in aqua regia). In the ash, Ca, K, Mg, Mo, P, V, and HM were measured according to DIN CEN/TS 15290 and 15297, respectively [51,52]. The DM, TOC (total organic carbon), pH, and EC of the ash were analyzed according to DIN CEN/TS 14774, ÖNORM EN 13137, DIN 38414-4, and DIN EN 13370 [53–56].

2.5. Microbiome Profiling

The soil DNA extraction and microbial community sequencing and analysis were performed for the soil samples collected at the end of the field trial in 2018. Soil DNA was extracted from 0.5 g of soil (previously stored at −20 °C and subsequently thawed prior to extraction) with the NucleoSpin® soil kit (Macherey-Nagel, Düren, Germany) in

accordance with the user manual. Microsynth GmbH (Balgach, Switzerland) performed the sequencing on an Illumina MiSeq using the 2 × 250 bp paired-end approach. For bacteria, the 16S rRNA gene (V4) was sequenced using primer pair 515 f and 806 r [57] (Caporaso et al., 2011). For fungi, the ITS2 region was sequenced using primer pair ITS3 and ITS4 [58].

Demultiplexed, quality-filtered, and trimmed sequences were analyzed in R version 4.2.0 [59] using package dada2 following the advised developers' protocol [60], eventually resulting in a bacterial and a fungal table of amplicon sequence variants (ASVs), giving their respective read counts per sample. Briefly, reads were filtered again using standard settings. Sequencing errors were inferred from each run and sequences were corrected for errors. Chimeric sequences were removed using removal Bimera Denovo. Each ASV was annotated regarding its best fit taxonomy using the UNITE reference (v2019) for the fungal and SILVA reference (v132) for the bacterial reads, respectively. Annotated ASV tables were used for a further statistical analysis.

2.6. Statistical Analyses

For the statistical data analysis and visualization, PAST software, version 4.07b, R (v. 4.0.2) and Microsoft Excel (Microsoft Office 365) were used [61,62]. Venn diagrams were produced with the web application Deepvenn [63]. In multivariate space, the impact of the different fertilization treatments on the entire sets of soil physicochemical and microbiological properties measured, respectively, as well as on the forage yield, forage quality, and plant coverage was evaluated by a permutational multivariate analysis of variance (PERMANOVA) [64] using the R package vegan (v. 2.6–4) [65]. Differences among sample groups were considered significant if *p*-values were smaller than 0.05. For testing the differences in single variables among treatments within the same year, an ANOVA was performed followed by a Tukey test. A principal component analysis (PCA) was used to compare the soil and forage variables among the sample groups. Prior to these analyses, the data were transformed (log transformation for soil physicochemical and forage data and Box–Cox for ASV tables) for normality assumptions. Normality was checked with the Shapiro–Wilk Test.

For plant cover, we analyzed the data using a generalized linear mixed model and the PROC GLIMMIX procedure in SAS 9.4. The model assessed the effects of treatment, year, and their interaction on grass, herb, and legume yield. Treatment and year were treated as fixed effects, and the interaction between these factors was also included to evaluate whether the impact of treatments varied across different years. To account for repeated measures across years within each plot, a compound symmetry (CS) covariance structure was implemented. This decision was informed by the CS structure's appropriateness for datasets with only two time points. Least squares means were calculated for each treatment and year, along with adjusted multiple comparisons using the Tukey–Kramer correction. The slice statement additionally enabled us to explore the interaction effects by analyzing the differences in treatment effects across different years and vice versa.

3. Results

3.1. Yield, Plant Cover, and Forage Quality

Yields tended to be lower in 2018 compared to 2014 (Figure 1) (mean difference = 1.0 t ha^{-1}; $p = 0.08$). In 2014 and 2018, the plots amended with CS, CSLime, CS + Lime, CSBMA, and CS + BMA had higher forage yields than the unamended plots and those receiving only lime or BMA (on average, 2.9 t ha^{-1} more in 2014, $p < 0.001$; and 2.6 t ha^{-1} in 2018, $p < 0.001$) (Figure 1a). Both grass and legume yield were significantly affected by treatment and year, without significant interactions. Herb yield was significantly affected by year but not by treatment (Table 1). Regarding the grass, all treatments receiving CS (alone or in combination with lime or BMA) showed similar yields. However, they differed from the unamended controls and the plots amended with only lime or BMA, respectively, in both years. For legumes, no significant differences were found, with the exception that both in 2014 and 2018, CS + BMA showed a significantly higher yield than the control. In addition,

the CS + BMA yield in 2014 was higher than for BMA alone, and in 2018, it was higher than the lime treatment (Table 1). Figure 2b shows an overview on the coverage of grass, legumes, and herbs for each of the three single cuttings in each year; no statistical analysis, however, has been made.

All forage quality parameters are given in Table S2, including digestibility of organic matter (dOM), metabolic energy (ME), and net energy lactation (NEL). Differences were detected between the years 2014 and 2018 ($p_{permanova}$ = 0.0001). However, no significant differences among the treatments regarding the forage quality (Table S3), neither in 2014 ($p_{permanova}$ = 0.5889) nor in 2018 ($p_{permanova}$ = 0.764) (see Table S3), were found.

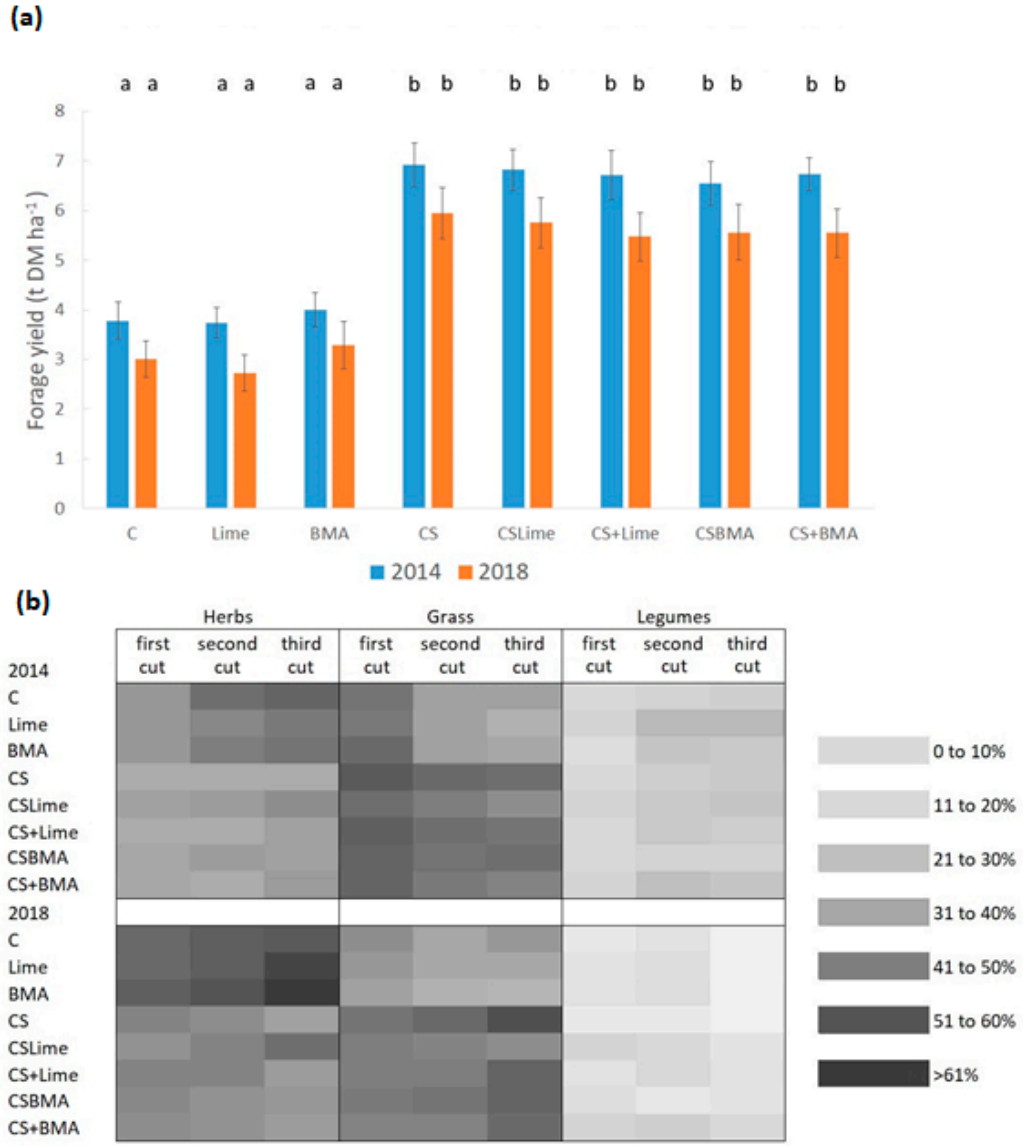

Figure 1. (**a**) Forage yield (t DM ha^{-1}) in 2014 and 2018 for the differently treated plots (mean ± SD, n = 4). Columns with the same lowercase letters are not significantly different according to PERMANOVA ($p \leq 0.05$) between treatments in the same year. (**b**) Forage coverage (%) separated into herbs, grasses, and legumes for the different fertilization treatments and for the years 2014 and 2018.

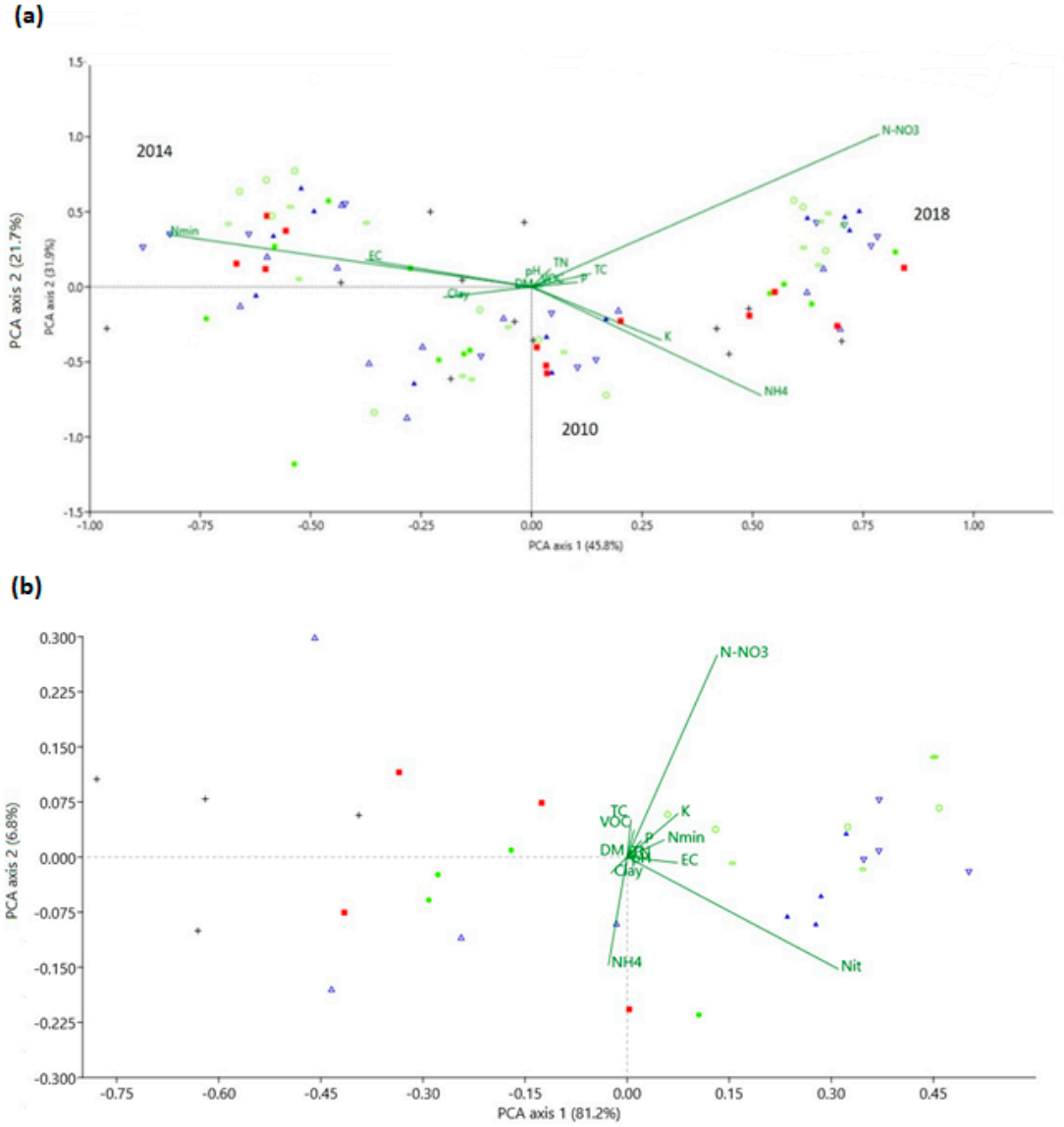

Figure 2. (**a**) The principal component analysis (PCA) of the soil physicochemical properties for the different treatments. (**b**) PCA of the soil physicochemical properties for the different treatments after eight years of soil amendment in 2018 (black cross: unamended control; green: lime (dot), CSLime (circle), CS + Lime (oval); blue: BMA (triangle), CSBMA (inverse triangle), CS + BMA (filled triangle); red: CS (filled square); DM = dry matter, VOC = volatile organic compound, EC = electric conductivity, Nit. = nitrification potential, TC = total carbon, TN = total nitrogen, P = phosphorus, K = potassium, N-NO_3^- = nitrate, NH_4 = ammonia).

Table 1. Total annual yield for grass, legumes, and herbs (t) for each treatment in 2014 and 2018. Mean values ± standard deviation of four replicates, different lowercase letters indicate significant differences within the years, and different capital letters indicate significant differences between the harvests of 2014 and 2018 ($p > 0.05$).

	Grass Yield (t DM ha^{-1} $Year^{-1}$)		Legume Yield (t DM ha^{-1} $Year^{-1}$)		Herb Yield (t DM ha^{-1} $Year^{-1}$)	
year	**2014**	**2018**	**2014**	**2018**	**2014**	**2018**
Treatment						
C	1.49 ± 0.20 aA	1.12 ± 0.24 aA	0.52 ± 0.05 aA	0.20 ± 0.09 aB	1.78 ± 0.23 aA	1.70 ± 0.18 aA
Lime	1.40 ± 0.17 aA	0.89 ± 0.17 aA	0.78 ± 0.15 abA	0.24 ± 0.05 aB	1.57 ± 0.31 aA	1.60 ± 0.56 aA

Table 1. *Cont.*

	Grass Yield (t DM ha^{-1} Year^{-1})		Legume Yield (t DM ha^{-1} Year^{-1})		Herb Yield (t DM ha^{-1} Year^{-1})	
year	**2014**	**2018**	**2014**	**2018**	**2014**	**2018**
BMA	1.64 ± 0.20 aA	0.99 ± 0.14 aB	0.63 ± 0.19 aA	0.32 ± 0.07 abB	1.73 ± 0.24 aA	1.99 ± 0.16 aA
CS	3.84 ± 0.35 bA	3.23 ± 0.69 bA	1.03 ± 0.23 abA	0.34 ± 0.16 abB	2.07 ± 0.28 aA	2.38 ± 0.40 aA
CS + BMA	3.39 ± 0.10 bA	2.64 ± 0.42 bB	1.27 ± 0.28 bA	0.90 ± 0.49 bB	2.17 ± 0.27 aA	2.21 ± 0.38 aA
CS + Lime	3.56 ± 0.49 bA	2.75 ± 1.05 bB	1.07 ± 0.32 abA	0.54 ± 0.34 abB	2.09 ± 0.36 aA	2.18 ± 0.33 aA
CSLime	3.09 ± 0.27 bA	2.52 ± 0.45 bA	1.11 ± 0.18 abA	0.67 ± 0.12 abB	2.34 ± 0.37 aA	2.38 ± 0.31 aA
CSBMA	3.57 ± 0.36 bA	2.77 ± 0.17 bB	0.91 ± 0.09 abA	0.57 ± 0.21 abB	2.25 ± 0.36 aA	2.21 ± 0.28 aA

3.2. Soil Physicochemical Properties

There was a clear differentiation of the samples along the first component with regard to the year of sampling. Nitrogen-related variables NH_4, $N\text{-}NO_3$, and N_{min} contributed the most to these differences along PC1 (Table 2; Figure 2a).

Table 2. Soil physicochemical properties of the differently treated soils showing means of the years 2010, 2014, and 2018 (n.d. = not determined; DM = dry matter, VOC = volatile organic compound, EC = electric conductivity, WHC = water holding capacity, Nit. = nitrification potential, TC = total carbon, TN = total nitrogen, P = phosphorus, K = potassium, Fe = iron, Mn = manganese, Cu = copper, Zn = zinc, B = boron, As = arsenic, Pb = lead, Cd = cadmium, Co = cobalt, Cr = chromium, Ni = nickel, Mo = molybdenum, Zn = zinc, V = vanadium, Hg = mercury). Soil cation exchange capacities are not shown. WHC and N-mineralization were not determined in 2010, and values of Zn are missing for 2014. CoVar% = mean coefficient of variation (%).

	Year	CoVar%	C	Lime	BMA	CS	CSLime	CS + Lime	CSBMA	CS + BMA
DM (%)	2010	1.5	78.2	78.3	77.8	77.0	77.3	77.4	76.8	76.7
VOC (%)	2010	8.3	6.8	6.3	6.5	7.4	6.2	6.2	6.6	6.8
pH ($CaCl_2$)	2010	2.1	5.2	5.3	5.2	5.2	5.4	5.3	5.2	5.3
EC (µS cm^{-1})	2010	13.3	28.5	35.5	34.5	37.5	38.3	37.5	37.0	44.0
NH_4 (µg N gDM^{-1})	2010	37.8	9.5	15.0	10.4	17.5	16.4	13.9	12.1	14.4
$N\text{-}NO_3$ (µg N g^{-1} DM)	2010	19.2	9.3	11.6	10.1	9.0	8.7	8.9	8.0	9.2
Nit. (ng N g^{-1} DM 5 h^{-1})	2010	45.2	102.6	56.8	64.2	119.9	111.9	82.5	75.0	98.3
TC (%)	2010	8.4	2.3	2.1	2.1	2.3	2.1	2.2	2.3	2.9
TN (%)	2010	13.4	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.3
P (mg kg^{-1})	2010	13.7	32.8	34.0	33.8	36.5	40.5	34.5	38.0	39.8
K (mg kg^{-1})	2010	23.9	60.5	81.5	76.3	89.8	96.0	105.0	87.0	109.3
Clay (%)	2010	5.5	17.0	17.5	18.0	17.0	17.5	17.0	18.5	17.0
Fe (mg kg^{-1})	2010	8.9	n.d.	n.d.	n.d.	255	259	240	228	253
Mn (mg kg^{-1})	2010	9.2	n.d.	n.d.	n.d.	240	242	236	222	238
B (mg kg^{-1})	2010	41.7	n.d.	n.d.	n.d.	0.0	0.1	0.1	0.0	0.1
As (mg kg^{-1})	2010	3.3	21.4	21.9	21.8	21.5	21.3	21.6	21.5	21.3
Pb (mg kg^{-1})	2010	6.9	27.8	26.5	27.6	28.0	25.9	26.7	27.4	28.4
Cd (mg kg^{-1})	2010	21.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Co (mg kg^{-1})	2010	3.5	15.2	15.5	15.7	15.2	15.5	15.6	15.1	15.1
Cr (mg kg^{-1})	2010	5.7	54.6	56.8	56.7	54.7	54.7	55.9	54.4	55.1
Cu (mg kg^{-1})	2010	9.2	38.7	40.7	40.7	39.1	40.6	40.5	39.2	40.0
Ni (mg kg^{-1})	2010	6.2	40.3	42.6	42.6	40.6	42.0	42.6	40.9	40.6
Mo (mg kg^{-1})	2010	9.0	0.9	0.8	0.8	0.9	0.8	0.8	0.9	0.9
Zn (mg kg^{-1})	2010	1.8	97.4	97.8	99.9	99.3	97.0	97.9	97.8	98.9
V (mg kg^{-1})	2010	2.8	53.0	53.0	53.8	53.0	51.3	51.5	50.9	52.5
Hg (mg kg^{-1})	2010	9.8	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
DM (%)	2014	1.8	74.2	73.1	73.2	72.8	72.9	72.8	73.0	72.4
VOC (%)	2014	23.6	5.6	6.6	6.5	7.2	7.1	7.2	7.4	6.4

Table 2. *Cont.*

	Year	CoVar%	C	Lime	BMA	CS	CSLime	CS + Lime	CSBMA	CS + BMA
pH ($CaCl_2$)	2014	1.9	4.9	5.1	5.2	5.1	5.2	5.4	5.3	5.3
EC ($\mu S\ cm^{-1}$)	2014	20.6	33.0	39.1	42.5	60.0	78.7	76.8	73.5	70.2
WHC (%)	2014	3.6	56.4	56.4	56.3	56.9	57.0	56.9	55.7	55.9
NH_4 ($\mu g\ N\ gDM^{-1}$)	2014	32.3	2.8	2.1	2.6	2.6	2.9	1.7	3.7	5.3
N-mineralization ($\mu g\ N\ g^{-1}\ DM\ d^{-1}$)	2014	14.8	10.2	10.5	14.6	14.6	13.3	14.3	15.5	12.5
N-NO_3 ($\mu g\ N\ g^{-1}\ DM$)	2014	25.4	14.4	13.7	15.9	21.7	30.5	28.1	27.1	39.9
Nit. ($ng\ N\ g^{-1}\ DM\ 5\ h^{-1}$)	2014	56.1	187	120	133	118	159	247	177	205
TC (%)	2014	28.5	2.3	2.4	2.3	2.5	2.5	2.3	2.5	2.8
TN (%)	2014	13.2	0.2	0.2	0.2	0.3	0.3	0.2	0.3	0.3
P ($mg\ kg^{-1}$)	2014	8.2	32.5	31.0	31.3	37.8	32.3	32.8	35.5	35.8
K ($mg\ kg^{-1}$)	2014	19.1	23.5	24.8	28.8	34.5	30.3	32.8	36.0	34.5
Clay (%)	2014	7.9	23.3	24.8	28.8	16.5	16.0	16.0	16.0	16.0
Fe ($mg\ kg^{-1}$)	2014	6.3	290	259	273	307	284	274	269	269
Mn ($mg\ kg^{-1}$)	2014	10.2	290	258	273	227	223	214	201	200
B ($mg\ kg^{-1}$)	2014	16.0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
As ($mg\ kg^{-1}$)	2014	2.7	20.7	21.0	20.5	20.5	20.9	20.4	21.0	20.6
Pb ($mg\ kg^{-1}$)	2014	11.5	24.3	22.7	25.0	24.7	21.7	23.8	23.4	23.1
Cd ($mg\ kg^{-1}$)	2014	4.5	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Co ($mg\ kg^{-1}$)	2014	3.7	16.2	16.7	16.5	16.0	16.6	16.4	16.2	16.1
Cr ($mg\ kg^{-1}$)	2014	5.0	59.2	60.8	59.7	57.8	60.5	59.6	57.9	58.1
Cu ($mg\ kg^{-1}$)	2014	9.7	37.7	39.9	38.8	37.9	39.9	39.5	38.0	38.7
Ni ($mg\ kg^{-1}$)	2014	5.5	47.7	50.2	49.6	47.6	50.4	49.6	47.8	47.6
Mo ($mg\ kg^{-1}$)	2014	6.4	1.1	1.0	1.0	1.1	1.0	0.9	1.0	1.0
V ($mg\ kg^{-1}$)	2014	2.7	57.7	57.2	57.3	57.3	57.9	57.2	56.1	56.6
Hg ($mg\ kg^{-1}$)	2014	9.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
DM (%)	2018	1.0	75.7	75.8	74.9	75.9	75.8	75.9	75.9	75.3
VOC (%)	2018	7.2	7.8	7.7	8.0	8.3	8.5	8.6	8.6	8.2
pH ($CaCl_2$)	2018	1.0	5.0	5.3	5.4	5.2	5.4	5.5	5.4	5.6
EC ($\mu S\ cm^{-1}$)	2018	8.9	15.5	22.3	24.0	23.5	30.8	27.0	26.8	30.0
WHC (%)	2018	4.4	35.6	34.6	34.5	31.5	35.3	36.1	35.6	34.8
NH_4 ($\mu g\ N\ gDM^{-1}$)	2018	13.4	9.6	10.6	10.8	10.9	9.5	7.8	8.5	8.7
N-mineralization ($\mu g\ N\ g\ DM^{-1}\ d^{-1}$)	2018	11.2	16.3	23.1	20.0	25.6	26.8	25.8	26.1	26.5
N-NO_3 ($\mu g\ N\ g^{-1}\ DM$)	2018	29.0	2.2	3.0	3.2	2.8	4.5	6.4	5.6	6.0
Nit. ($ng\ N\ g^{-1}\ DM\ 5\ h^{-1}$)	2018	35.3	155	429	306	336	872	954	786	1059
TC (%)	2018	10.9	3.4	3.0	2.7	3.2	3.6	3.0	3.4	3.4
TN (%)	2018	14.7	0.3	0.3	0.2	0.3	0.4	0.3	0.3	0.3
P ($mg\ kg^{-1}$)	2018	10.8	40.5	37.5	35.5	43.0	39.0	42.0	44.3	49.0
K ($mg\ kg^{-1}$)	2018	9.8	48.3	51.0	50.0	67.8	75.5	81.0	79.5	81.0
Clay (%)	2018	5.8	14.0	12.5	12.5	12.5	12.0	11.5	12.0	12.0
Fe ($mg\ kg^{-1}$)	2018	8.3	314	282	282	338	315	298	291	300
Mn ($mg\ kg^{-1}$)	2018	10.7	217	207	210	246	240	230	220	219
B ($mg\ kg^{-1}$)	2018	14.8	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.3
As ($mg\ kg^{-1}$)	2018	2.3	23.4	23.4	22.7	23.0	23.2	23.1	23.0	23.0
Pb ($mg\ kg^{-1}$)	2018	8.3	28.5	26.2	25.4	27.7	25.0	24.6	26.3	27.6
Cd ($mg\ kg^{-1}$)	2018	5.0	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Co ($mg\ kg^{-1}$)	2018	3.8	19.0	19.3	19.2	18.9	19.3	19.3	18.6	18.9
Cr ($mg\ kg^{-1}$)	2018	5.8	65.3	65.7	65.8	62.9	64.8	63.8	61.7	63.0
Cu ($mg\ kg^{-1}$)	2018	8.4	45.0	46.5	46.1	44.6	51.1	46.1	44.2	45.8
Ni ($mg\ kg^{-1}$)	2018	6.6	49.4	51.5	51.2	48.3	50.9	50.6	48.2	48.3
Mo ($mg\ kg^{-1}$)	2018	11.5	1.3	1.2	1.2	1.3	1.3	1.3	1.5	1.3

Table 2. *Cont.*

	Year	CoVar%	C	Lime	BMA	CS	CSLime	CS + Lime	CSBMA	CS + BMA
Zn (mg kg^{-1})	2018	1.6	126	121	122	125	126	123	122	125
V (mg kg^{-1})	2018	1.7	69.4	68.5	68.2	68.5	68.0	66.2	65.1	67.3
Hg (mg kg^{-1})	2018	17.0	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1

A clear clustering of fertilization treatments was observed for the soil samples collected at the end of the trial in 2018 (Figure 2b). Samples receiving no or inorganic fertilizers and solely CS clustered along the negative side of PC1, while the samples amended with CS in any combination with lime or BMA clustered on the positive side of PC1. The variables Nit, $N\text{-}NO_3$, and EC contributed the most to these differences along PC1. Concerning PC2, the most contributing variables included Nit, $N\text{-}NO_3$, and NH_4 (Figure 2b), reaching higher values in the treatments CSBMA, CS + BMA, CSLime, and CS + Lime compared to the unfertilized control and the plots receiving solely lime, BMA, or CS. More specifically, Nit values were on average 600 ng N g^{-1} DM 5 h^{-1} higher, while values for $N\text{-}NO_3$ and EC were 2.8 µg $N\text{-}NO_3$ g DM^{-1} and 7.5 µS cm^{-1} higher, respectively (Table S2). Average NH_4 concentrations were higher in treatments receiving solely one additive. The average values were 1.1 µg N g DM^{-1} higher compared to the unfertilized control and 2.1 µg N g DM^{-1} higher than in plots receiving CS in any combination with BMA or lime (Table 2).

Overall, the soil physicochemical properties differed significantly among the years 2010, 2014, and 2018 ($p_{permanova}$ = 0.0003). We observed an increase in VOC, TOC, TN, $N\text{-}NO_3$, and EC from 2010 to 2014 followed by a decrease in 2018. Over time, both pH and P content slightly increased (Table 2, *p*-values in Table S1). In addition, our results showed a slightly enhanced pH in soils receiving any treatment with the addition of BMA or lime from 2010 to 2018 (0.04–0.28 pH units), while the pH decreased slightly in the unfertilized control (0.23 pH units). In the plots receiving only one additive, pH decreased with lime and CS (0.07 and 0.04 pH units) and increased with BMA (0.18 pH units) from 2010 to 2018 (Table 2). These changes in pH were significant among all treatments (except CS) and the unfertilized control. Phosphorus increased for all plots except for CSLime from 2010 to 2018. The increase was about 1.75–9.25 mg kg^{-1}, while the decrease in CSLime was 1.5 mg kg^{-1} (Table 2). The plots only receiving BMA had a lower P content (on average, 11.2 mg kg^{-1} lower) compared to CS, CSLime, and CSBMA, which were similar. However, these differences were only significant comparing CSBMA with the unfertilized control and those plots treated with only lime/BMA (on average, 16.4 mg kg^{-1} lower) from 2010 to 2018. Comparing 2010 and 2018, the concentrations of VOC, TOC, and TN increased in all of the treatments by 0.97–2.32%, 0.48–1.51%, and 0.04–0.19%, respectively (except for TN in CS + BMA, which did not change) (Table 2).

Neither the time of sampling nor the type of application had a significant impact on the HM contents (Table 2), corroborating hypothesis (iv).

3.3. Microbial Properties and Community Composition

In 2010, BR and C_{mic} reached higher values in the plots amended with CS and CSBMA than in those receiving only BMA and lime (Figure 3a,b). However, neither BR nor C_{mic} differed among the fertilization treatments in 2014 (Figure 3a,b). In 2018, the combination of CS with either BMA (CSBMA and CS + BMA) or lime (CSLime and CS + Lime) led to an increase in C_{mic} compared to BMA-, lime-, and CS-treated plots (Figure 3b). In contrast, lower respiration rates were recorded in CSLime and CSBMA treatments at the end of the trial (Figure 3a). Basal respiration and C_{mic} showed an increasing trend over the years for most of the fertilization treatments (Figure 3a,b), while the highest qCO_2 was observed in 2010 (Figure 3c).

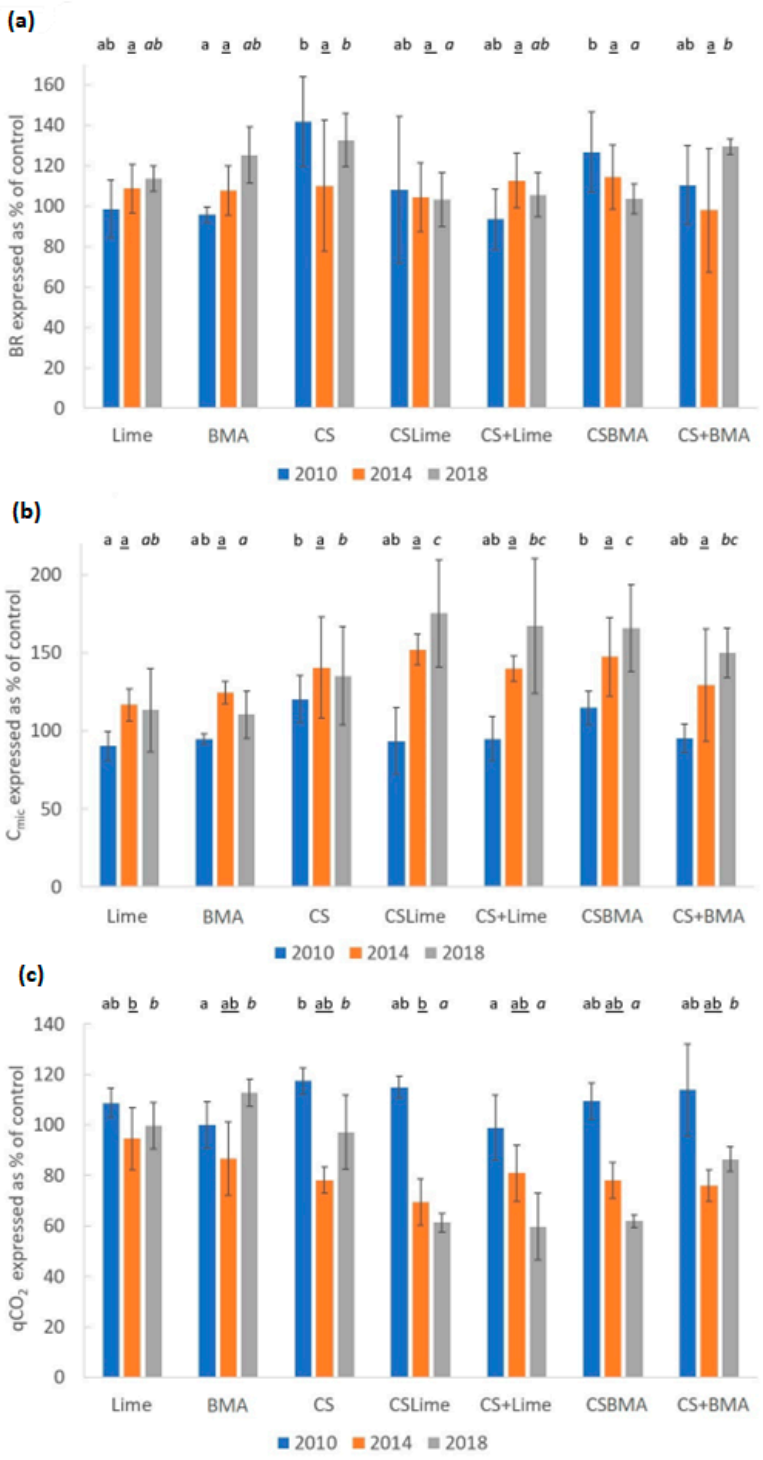

Figure 3. Percentages (controls are equal to 100% for each of the years) for (**a**) basal respiration (BR; μg CO_2 g^{-1} soil (dry weight, dw) h^{-1}), (**b**) microbial biomass carbon (C_{mic}; μg C g^{-1} soil dw), and (**c**) metabolic quotient (qCO_2; mg CO_2–C mg^{-1} C h^{-1}) of the different years and treatments (mean ± SD, n = 4). In the control treatment, BR values were 1.66 μg CO_2 g^{-1} soil dw (2010), 2.20 μg CO_2 g^{-1} soil dw (year 2014), and 8.85 μg CO_2 g^{-1} soil dw (2018). In the case of C_{mic}, the values were 432.79 μg C g^{-1} soil dw (2010), 511.2 μg C g^{-1} soil dw (2014), and 1294 μg C g^{-1} soil dw (2018). qCO_2 had values of 3.83 (2010), 4.29 (year 2014), and 6.9 (year 2018). Columns with the same lowercase letters are not significantly different among treatments within the same year, and standard letters = 2010; underlined letters = 2014; and italic letters = 2018 ($p \leq 0.05$).

Regarding the bacterial community composition, post hoc tests did not indicate differences among the plots that had received CS in any combination with lime or BMA (Table S4). They, however, differed from the plots that had not received CS or CS alone, except for BMA and CS + BMA (Table S5). Control, BMA, and CS treatments also differed from each other (Figure 4a). The Shannon's diversity indices with standard deviation (n = 4) for the different treatments were Shannonc = 6.15 ± 0.28; $Shannon_{Lime}$ = 6.18 ± 0.13; $Shannon_{BMA}$ = 6.51 ± 0.09; $Shannon_{CS}$ = 6.37 ± 0.09; $Shannon_{CS+Lime}$ = 6.28 ± 0.08; $Shannon_{CS+BMA}$ = 6.38 ± 0.13; $Shannon_{CSLime}$ = 6.43 ± 0.18; and $Shannon_{CSBMA}$ = 6.31 ± 0.09.

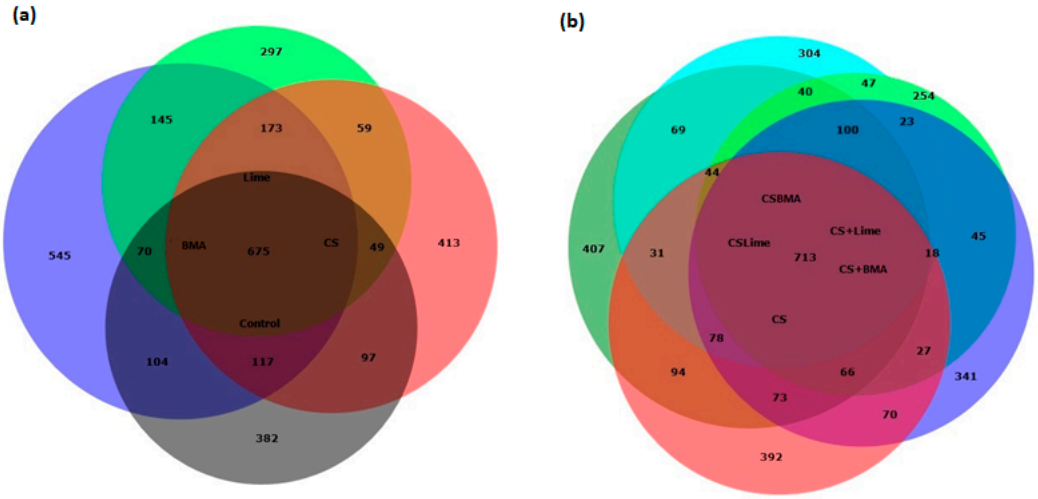

Figure 4. Core, shared, and unique ASVs of the bacterial community in the control, BMA, lime, and cattle slurry (CS) plots (**a**), and those that received CS alone or in any combination with BMA or lime (**b**). Venn diagrams drawn with DeepVenn [61].

Proteobacteria (32.7% of all ASVs), Actinobacteria (25.6% of all ASVs) and Acidobacteria (17.9% of all ASVs) comprised the most abundant phyla in all the treatments (Table S5). *Nitrosomonadaceae* (Proteobacteria) and *Acidothermaceae* (Actinobacteria) were amongst the ten families mostly found in the control plots (10th most, 104 ASVs) and in those treated with lime (9th most, 118 ASVs), BMA (9th most, 142 ASVs), and CS (10th most, 137 ASVs), and to a lower extent in the plots amended with CS + BMA (15th most, 112 ASVs), CS + Lime (26th most, 87 ASVs), CSLime (22nd most, 111 ASVs), and CSBMA (14th most, 107 ASVs). In contrast, Bacillales (Firmicutes, family level could not be detected by sequencing) and *Peptostreptococcaceae* (Firmicutes) were mostly found in soils amended with any of the treatments containing CS (on average, 151 ASVs in the treatments containing CS versus 25 ASVs in the treatments without CS regarding Bacillales and 14 ASVs versus 127 ASVs for *Peptostreptococcaceae*, respectively).

In the control plots along with those amended with either only BMA, lime, or CS, 3126 ASVs could be detected; 21.6% of these ASVs were shared among these treatments. Meanwhile, 12.2%, 17.4%, 9.5%, and 13.2% bacterial ASVs were unique in each of the abovementioned treatments (Figure 4a). In the plots treated with CS alone or in any combination with BMA or lime, a total of 3236 bacterial ASVs were detected and 22% of them were detected across all these treatments. Plots treated with CS had 12.1% unique bacterial ASVs, while in CSBMA, 9.4%; CS + BMA, 10.5%; CSLime, 12.6%; and CS + Lime, 7.8% unique ASVs were observed (Figure 4b).

Concerning the fungal microbiome, *Cladosporidaceae* (Ascomycota), *Nectriaceae* (Ascomycota), and *Piskurozymaceae* (Basidiomycota) were amongst the ten families most frequently found in all of the treatments (on average, 539, 564, and 397 fungal ASVs, respectively; Table S5). However, the family *Lasiosphaeriaceae* (Ascomycota) was only detected in those plots that had received CS (396 ASVs on average). High abundances of *Marasmiaceae* (253 ASVs) and *Ceratobasidiaceae* (236 ASVs), both Basidiomycota, were found in the control plots. *Marasmiaceace* were absent in all the other treatments, while *Ceratobasidiaceae*

were only found in lime (41 ASVs), CS (19 ASVs), and CSLime (63 ASVs), although in low abundances. The Basidiomycota *Bolbitiaceae* were exclusively found in soils treated with only BMA (225 ASVs). Furthermore, *Mycosphaerellaceae* and *Leptosphaeriaceae* (both Ascomycota) were highly abundant in plots treated with solely BMA (399 and 255 ASVs, respectively), and in lower abundance in the others (53 and 11 ASVs on average, respectively). In soils amended solely with CS, *Clavicipitaceae* (Ascomycota) was the most abundant family (1109 ASVs), which was absent in CS + Lime- and CSLime-treated soils. A high abundance of *Pseudorobillarda* spp. (Pleosporales, Ascomycota) was detected in CS + BMA (379 ASVs) and CSBMA (101 ASVs), whilst it was absent in the control and the lime-treated soils and it appeared in low abundances in soils amended with BMA, CS + Lime, and CSLime (on average, 30 ASVs). Pleosporales *incertae familiae* were among the 10 most abundant families in CSLime plots (353 ASVs), and in those amended with CS (382 ASVs). However, they were in low abundance in the control plots (4 ASVs), and absent in those receiving only lime and BMA. Furthermore, CSLime plots had the highest abundance (269 ASVs) for *Plectosphaerellaceae* (Ascomycota), while their abundance was 4–9 times lower in all the other treatments. *Clavicipitaceae* (Ascomycota) in the CSBMA plots (1109 ASVs) were 2–7 times more abundant than in the other treatments. The family *Amorosiaceae* (Ascomycota) was also found in high abundance in CSBMA-treated soils (331 ASVs). The mean Shannon's diversity indices with standard deviation (n = 4) for the different treatments were Shannon = 4.99 ± 0.08; $Shannon_{Lime}$ = 4.95 ± 0.08; $Shannon_{BMA}$ = 5.1 ± 0.19; $Shannon_{CS}$ = 4.71 ± 0.32; $Shannon_{CS+Lime}$ = 4.84 ± 0.05; $Shannon_{CS+BMA}$ = 4.92 ± 0.22; $Shannon_{CSLime}$ = 4.73 ± 0.36; and $Shannon_{CSBMA}$ = 4.96 ± 0.10.

In the control, BMA, lime, and CS plots, 1843 fungal ASVs were detected, of which 15.6% were shared among all treatments, while they each had 13.2%, 17.5%, 14.6%, and 15.1% unique fungal ASVs, respectively (Figure 5a). Treatments, which were solely amended with CS or any combination with BMA or lime, had 1713 ASVs in total and shared 16.1% of them (Figure 5b). In CS, 12.6% unique fungal ASVs were detected; and CSBMA had 14.1%; CS + BMA, 12.7%; CSLime, 12.6%; and CS + Lime, 8.8% unique fungal ASVs (Figure 5b).

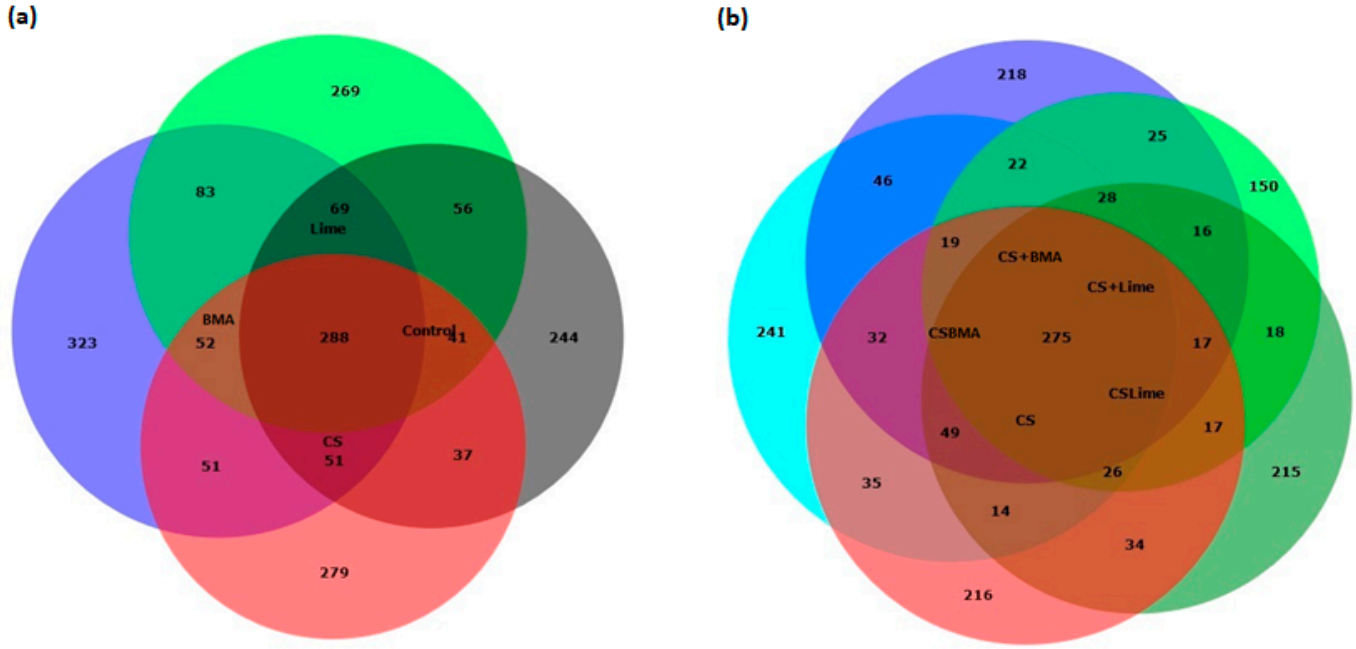

Figure 5. Venn diagrams showing the core, shared, and unique ASVs of the fungal community of control, BMA, lime, and CS plots (**a**) and plots that received CS alone or in any combination with BMA or lime (**b**). Venn diagrams drawn with DeepVenn [61].

4. Discussion

4.1. Yield, Plant Cover, and Forage Quality

We observed that the yields in 2014 were higher than those in 2018. This is most likely due to the lower precipitation during the growing season in 2018 [62,63].

Our results corroborate the review by Ram and Masto [64], stating that the mixture of biomass ash with CS resulted in increased plant yields. In our study, the application of CS with BMA led to significantly higher forage yields (2.7 t ha^{-1}) compared to the unamended control and plots solely receiving BMA (Figure 1). In more detail, our findings suggest that CS is the main factor enhancing forage yield, whereas the addition of BMA/lime only played a minor role. This was probably related to our soils being poor in N, making N the limiting factor for plant growth (Table 2). This hypothesis is also supported by the fact that the amount of TN was higher in soils amended with any variant of CS (CS, CSLime, CS + Lime, CSBMA, CS + BMA; Table 2) and also by the higher yields observed in these plots (Figure 1a). This is also corroborated by the enhanced nitrogen mineralization rate observed for all plots that had received CS (Table 2).

In our study, the mixture of BMA and CS did not increase the forage yield over the yield produced in CS plots (Figure 1b). This is in contrast to Bougnom et al. [20] who observed significantly higher forage yields when BMA was mixed with CS compared to CS alone. These discrepancies might have two reasons: First, in their greenhouse study, an amount of 3 t ha^{-1} of BMA was applied, in contrast to the dosage of 0.5 t ha^{-1} here. Second, their soils were poorer in nutrients compared to the soil in this study. Here, the soil initially had a good status of micronutrients (Table 2), and thus little benefit of BMA could be expected. In general, the most important effect of lime and ash application is a pH increase, which occurred in this experiment (comparing soil pH among treatments each year). However, the soil was probably not acidic enough to reflect this effect on grassland growth. From these findings, we derive that BMA only has an effect on forage yield in soils with an initially poor nutrient status and a low pH. Hypothesis (i) stating that BMA may serve as a substitute for traditional lime may thus be accepted; however, an enhancement of forage yield together with and without the use of cattle slurry has not been found as true.

Nevertheless, the addition of ash may lead to pollution with HMs and thus pose a risk [14,65]. In the case of this study, however, none of the HMs exceeded the thresholds published by Toth et al. [66] irrespective of the year of sampling (Table 2), and thus hypothesis (v) can be accepted. Consequently, HM concentrations should be monitored in case of continued application. However, a benefit of biomass application to CS treatment could be odor reduction, as this has been shown to occur if hot BMA is used as an additive or when BMA is added during composting processes [67–69].

While some of the forage quality indicators varied among the different years, no significant differences were observed regarding the treatments. Our findings show that herbs grow stronger in unfertilized soils or soils amended only with BMA/lime, which is in line with Schellberg et al. [70]. In addition to BMA application, the influence of CS on the forage composition is of interest. Our results showed that grass grew better on plots receiving CS with or without the combination with BMA/lime. Therefore, it can be concluded that CS enhanced the growth of grass through the provision of rapidly available nitrogen. Hence, the ratio of herbs and grass on total plant coverage raised from 2014 to 2018 while the percentage of legumes decreased. The decrease in legumes can be explained through the drier conditions in 2018 and the ability of gramineous plants to better withstand drought stress [63,69]. These findings are in accordance with the results of Pirhofer-Walzl et al. [70]. These authors also detected differences in the forage composition regarding different growth periods and years and they showed that CS application increases the amount of grass. However, they found only a small effect of CS on forage quality [70]. These results are in line with our findings. The reason for a lower effect of CS on forage quality could be the low mineral requirements of grasses, which was likely covered in this experiment. The fact that CS provided N and P could explain the poor behavior of legumes, which were probably limited by P availability in the treatments without CS and unable

to compete with grasses in the treatments with CS. The overall forage quality showed no significant difference among the treatments. An additional explanation for the similar forage quality could also be that the minerals bound in the slurry are taken up more slowly by the plants than simple ions [71–73].

4.2. Soil Physicochemical Properties

After eight years of amendment, enhanced concentrations of Nit, VOC, EC, and N-NO_3 were detected in soils treated with CS compared to the start of the experiment (Table 2). This supports that long-term fertilization exceeds the beneficial effects of short-term fertilization. These findings are in line with those summarized by Ram and Masto [64]. Unexpectedly, only small changes in soil pH were found following the different fertilization treatments, despite the initial soil pH being acidic (Table 2) and the BMA having had a pH close to 12 (Table S1). This finding contradicts a previous study [74], finding an increase of up to two pH units when agricultural soils were amended with CS and BMA. Therefore, it seems that the soil in the present study is either capable of buffering pH changes and/or the neutralizing capacity of the ash was different to that used by Aboltins et al. [74], who, however, used high single dosing.

Furthermore, no significant differences of the overall physicochemical soil properties were observed comparing soils solely amended with BMA or lime. Moreover, no differences among CSBMA/CS + BMA and CSLime/CS + Lime were detected regarding all the physicochemical properties measured. These findings show that traditional liming agents can be substituted with BMA. Even in a long-term scale, no effects of BMA application were found when compared to traditional liming.

4.3. Microbial Properties and Community Composition

The advantage of the combined application of CS and BMA/lime is supported by the enhanced total C_{mic} and the decrease in qCO_2 at the end of the trial (Figure 3a,c). This points towards a lower stress level for the soil microbial community compared to the control plots [75]. Our results suggest that the supply of nutrients following the application of a combination of CS and BMA/lime is beneficial for the microbiota, as reflected by the lower stress level. The explanation for this phenomenon could be that on the one hand, a broader range of nutrients is available through the application of both types of fertilizers and on the other hand that both organic and inorganic fertilizers influence the microbial activity and community in different manners [76].

In our study, neither the bacterial nor the fungal diversity differed among treatments; still, the composition of the microbiota varied among the differently treated plots (Figures 4 and 5). For the bacterial community, this differentiation seemed to be driven by the sole addition of BMA, lime, and CS in different ways, as indicated by the appearance of unique bacterial ASVs in these soils. The combination of CS with either lime or BMA did not result in a significant difference. For the fungal communities, the main driver of compositional differences among sample groups was the application of CS. Furthermore, our results showed that the fungal community was altered to a larger extent than the bacterial one. This assumption is based on the finding that in the fungal community, core ASV numbers were comparable to the unique ones (Figure 5).

Previous studies showed that an alteration of plant coverage on fertilized soils can have strong influences on the microbial community [77,78]. Therefore, it is plausible that the differences in plant coverage among treatments (Figure 1) may have influenced the soil microbial community composition. In line with previous studies [78–82], we observed that the application of CS in combination with lime or BMA led to significant changes in the soil microbial community composition when compared to treatments solely receiving one type of fertilizer (Figures 4 and 5). However, whether lime or BMA was used for the mixture did not have a discriminating effect on the microbial community. Therefore, it appears that the application of a combination of organic and inorganic fertilizer is more important for both soil and microbiological soil properties than the choice of inorganic fertilizer.

In line with our findings, previous studies also pointed out the advantages of liming on microbial communities. Several studies conclude that liming has an overall positive impact on soil microbiota [83–85]. In contrast, in a short-term trial, Neilson et al. and Yin et al. [86,87] could not detect an effect of liming on the soil microbial community. They showed that the interplay of the microbiota, plants, and physicochemical properties cannot be separated from each other and that changes in either one of these factors alone are insufficient to describe alterations of another factor. This hypothesis is further underlined by the heterogeneity of soils in general and the resulting heterogeneity of microbial communities [88–92]. Our results show that the addition of either BMA or lime to CS altered the microbial community compared to no fertilization and the sole addition of BMA or lime. Nonetheless, the addition of either lime or BMA alone did not lead to significant differences in the microbial community composition. This also holds if CS is mixed with either lime or BMA; no significant differences could be observed between these two treatments, and thus hypotheses (ii) (apart from some compositional changes in the microbiota) and (iii) have to be rejected. These findings suggest that traditional liming agents can be substituted with BMA if they are added alone or with the same organic fertilizer.

5. Conclusions

Over the course of an eight-year field trial on grassland, we investigated the influences of BMA, $CaCO_3$, and CS as well as combinations thereof on yield, forage quality, and plant coverage, and on soil physicochemical properties, and the composition and diversity of microbial communities. We conclude that carbonated lime ($CaCO_3$), a classic liming agent used on grassland, can be substituted by BMA since its addition showed the same effects on forage yield and microbial properties as observed for $CaCO_3$ addition. Despite that many of the evaluated properties did not differ between CS alone or combined with $CaCO_3$ or BMA, some trends differentiating soil properties (pH, Nit) could be an indication that in the long term, the combined amendments represent an improvement.

Of further interest for farmers is the way the fertilizers are applied. The previous mixture (should be performed shortly before application to the field to prevent sedimentation of the ash in the tanks) of CS and lime or BMA can be regarded as favorable for a farmer, because one field application suffices. The way of application did not affect those properties directly relevant for farming, including yields, forage quality, and plant composition.

However, the soil microbiota reacted with shifts in bacterial and fungal communities' composition more sensitively than the chemical properties did. Still, any farming practice concerning combined or the separate application of CS and BMA/$CaCO_3$ appears to be suitable.

As long as the ashes meet quality requirements and the amounts used remain below the legal limits, grassland-based recycling of BMAs appears to be advisable. Further studies involving different soil types, in particular acidic soils, are recommended. Altogether, our findings broaden the knowledge on the use of BMAs for the amelioration of grasslands, and thus contribute to a circular economy.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14071568/s1, Table S1: Properties of the biomass ash and cattle slurry used for amending the soils. Further depicted are the Austrian legal thresholds for agricultural use; Table S2: $P_{Tukey's}$-Values of the physicochemical soil parameters regarding the different treatments over all sampling points (2010–2018). $P_{Tukey's}$-values < 0.05 were considered significant; Table S3: *p*-Values of the bacterial community composition regarding the different treatments. *p*-values < 0.05 were considered significant; Table S4: Depiction of the 10 most abundant bacterial families found in the differently treated plots in 2018. From top to bottom the ASV numbers are sorted according to the ASV count for each treatment. The list below shows to which kingdom, phylum, class, order, family and genus the depicted ASV numbers belong; Table S5: Depiction of the 10 most abundant fungal families found in the differently treated plots in 2018. From top to bottom the ASV numbers are sorted according to the ASV count for each treatment. The list below shows to which kingdom, phylum, class, order, family and genus the depicted ASV numbers belong.

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