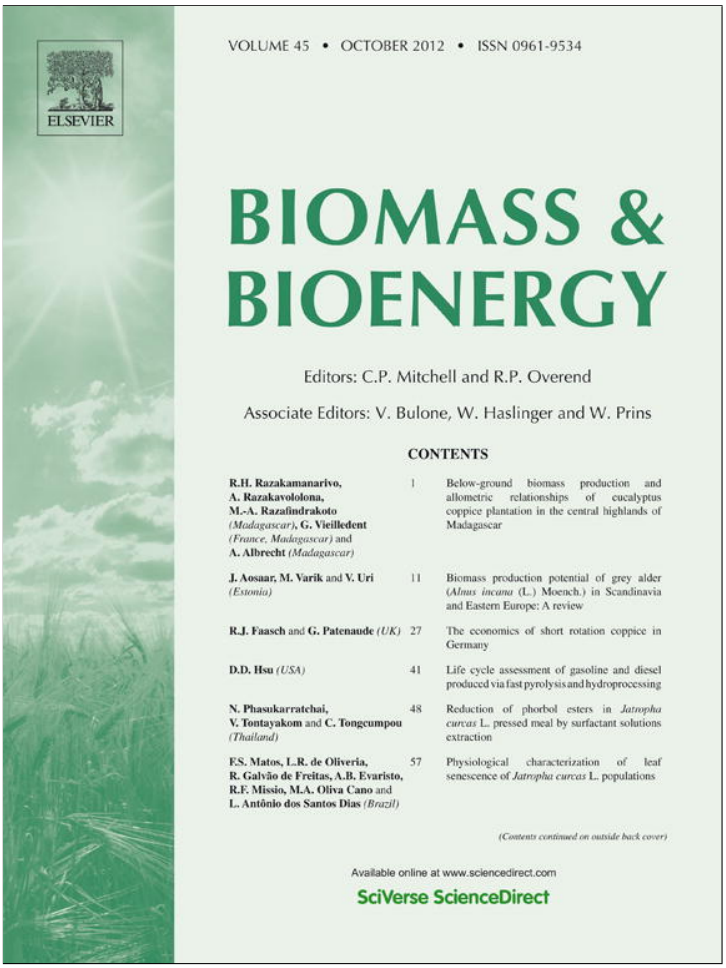

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Decomposition of biogas residues in soil and their effects on microbial growth kinetics and enzyme activities

Ruirui Chen [a,b], *Evgenia Blagodatskaya* [c,d,e,*], *Mehmet Senbayram* [b,f], *Sergey Blagodatsky* [g,c], *Olga Myachina* [h], *Klaus Dittert* [b], *Yakov Kuzyakov* [e]

[a] *State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, 210008 Nanjing, China*
[b] *Institute of Plant Nutrition and Soil Science, Christian-Albrecht University Kiel, 24118 Kiel, Germany*
[c] *Institute of Physicochemical and Biological Problems in Soil Science, Russian Academy of Sciences, 142290 Pushchino, Russia*
[d] *State Key Laboratory of Atmospheric Boundary Layer Physics and Atmospheric Chemistry, Institute of Atmospheric Physics, Chinese Academy of Sciences, 100029 Beijing, China*
[e] *Department of Soil Science of Temperate Ecosystems, Büsgen-Institute, University of Göttingen, 37077 Göttingen, Germany*
[f] *Institute of Plant Nutrition and Environmental Sciences, Hanninghof Research Center, 48249 Duelmen, Germany*
[g] *Institute of Plant Production and Agroecology in the Tropics and Subtropics, University of Hohenheim, 70593 Stuttgart, Germany*
[h] *Institute of General and Inorganic Chemistry, 100170 Tashkent, Uzbekistan*

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ABSTRACT

The rapid development of biogas production will result in increased use of biogas residues as organic fertilizers. However, control of microbial activity by organic fertilizers remains a challenge for modern land use, especially with respect to mitigating greenhouse effects and increasing C sequestration in soil. To address this issue, we compared CO_2 emissions, microbial growth and extracellular enzyme activities in agricultural soil amended with biogas residues (BGR) versus maize straw (MST). Over a 21 day incubation period, 6.4% of organic C added was mineralised and evolved as CO_2 with BGR and 30% with MST. As shown by the substrate-induced growth respiration approach, BGR and MST significantly decreased the specific microbial growth rate (μ) and increased the microbial biomass C in the soil, indicating a clear shift in the microbial community to slower-growing microorganisms. Because of the reduced availability of C associated with the less labile C and more lignin in biogas residues, observed μ values and microbial biomass C were lower after BGR application than after MST application. After 21 days incubation, BGR had no effect on the activity of three extracellular enzymes: β-glucosidase and cellobiohydrolase, both of which are involved in cellulose decomposition; and xylanase, which is involved in hemicellulose decomposition. In contrast, MST significantly increased the activity of these three enzymes. The application of biogas residues in short-term experiment leads to a 34% increase in soil C content and slower C turnover as compared to common maize residues.

* *Corresponding author*. Institute of Physicochemical and Biological Problems in Soil Science, Russian Academy of Sciences, 142290 Pushchino, Russia.
E-mail address: sblag@mail.ru (E. Blagodatskaya).
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Abbreviations

BGR	biogas residues
T_{lag}	lag period
MST	maize straw
MB	microbial biomass
GHGs	greenhouse gases
AMB	active microbial biomass
SOM	soil organic matter
AMC	L-leucine-7-amino-4-methyl coumarin
MUF	4-methylumbelliferyl-β-D-glucopyranoside
LAP	leucine amino peptidase
SIGR	substrate-induced growth response
PLFA	phospholipid-derived fatty acids
PE	priming effect

1. Introduction

The European Union endorsed in 2009 a mandatory target of a 20% share of energy from renewable sources in overall European Community energy consumption by 2020 [1]. Biomass energy from plants will play a major role in the substitution of fossil fuels with renewable resources [2]. Biogas is an emerging renewable energy source which derives from the conversion of plant biomass and organic waste into biofuels through anaerobic decomposition. Over the last decade, the number of biogas plants has significantly increased in industrialised regions, especially in Europe [3], and maize stover has become the most dominant energy crop for biogas production in Central Europe [4,5]. This rapid development of biogas production will result in increased production of biogas residuals (BGRs) with the concomitant utilisation of BGRs as organic fertilizers within agriculture [6].

Consistent with other organic fertilizers, BGRs enhanced crop yield [7,8] and improved N uptake, improved soil structure and water-/nutrient-binding capacities [9]. However, BGRs decomposition in soils after their application and the associated effects on soil C turnover have not been as extensively evaluated as for other organic fertilizers. Generally, organic fertilizers can efficiently increase the organic C content in soils [10]. However, it has been reported that intensive application of organic fertilizers will contribute to extra CO_2 [11] evolution by enhancing soil C turnover [12], thereby contributing to and possibly accelerating greenhouse effects. In particular, biogas residues contain high concentrations of ammonium N (50–75% of total N) [10], which is limited in common plant residues, e.g. maize straw. Ammonium N was reported to have a stimulating effect on the decomposition of plant residues and native soil organic matter [13]. Therefore, untreated maize stover and fermented residues from maize straw (BGRs) are compared in the current soil incubation experiment, to improve sustainable management of C involved in the newly-used BGRs in agro-ecosystems.

Soil microbial biomass has been recognised as the driving force for residues mineralization in soils, although it usually comprises only about 1–3% of total soil organic carbon [14]. The extracellular enzymes, which are biological catalyst of specific reactions, play a key role in the decomposition of native and exogenous organic matters in soils [15], and thus regulate its turnover and C flows in soils. Both respond quickly and sensitively to the changes in agricultural management [16], and can be considered as good markers of soil biological processes. It is generally accepted that application of organic fertilizers stimulates soil microbial biomass, basal respiration [17] and enzyme activities [18]. In contrast, Makadi et al. [19] showed that BGRs application has not caused drastic changes in soil microbiological properties, including invertase, dehydrogenase, catalase activities and the number of different groups of soil microbes by plate dilution technique. Toxicity caused by some trace contaminants during the anaerobic fermentation, such as phenolic compounds, chlorinated paraffins and polycyclic aromatic hydrocarbons [20,21], may even have negative effects on microbial mediated decomposition of BGRs in soils. The limited number of studies does not allow drawing consequences concerning soil C turnover after applying BGRs to soils, especially on the changes of microbiological properties in soils. Therefore, understanding the underlying microbiological and biochemical features related to soil C cycle after BGRs application, is urgently needed dealing with future challenges in the use of BGRs. To address this, an experiment under controlled conditions was set up: 1) to evaluate the mineralization dynamics of BGRs and its impact on the turnover of soil C; 2) to compare the effects of BGRs with conventional maize residues on microbial growth kinetics and extracellular enzyme activities in the soil; and 3) to explore the mechanisms of soil C turnover change after BGRs application by linking functional properties of microbial community with decomposition patterns.

2. Material and methods

2.1. *Experimental design*

Soil used in these experiments was collected from the upper 20 cm of the Hohenschulen experimental farm of Kiel University (10.0°E, 54.3°N), northern Germany. The soil is classified as Stagnic Luvisol, with a sandy loam texture, a total C content of 1.5%; pH 6.5 (0.01 mol dm^{-3} $CaCl_2$ 1:4), and a water holding capacity of 310 g kg^{-1}. Before use, the soil was air-dried, homogenised and sieved <2 mm. Roots and other plant residues were carefully removed.

The pot experiment with 3 replicates included (1) a non-amendment soil (Control); (2) soil amended with biogas residues (BGR); and (3) soil amended with maize straw and mineral N (MST). Aboveground parts of maize (*Zea mays* L.) were harvested from Hohenschulen experimental farm of Kiel University, dried at 60 °C, crushed with grinder and homogenised, then stored under dry conditions before use. Biogas residues were produced at an agricultural biogas plant in Marienthal, Northern Germany, from maize plants which were fermented at 40 °C for 65 days. The residual fermentation

effluents collected from the tank were used directly as biogas residues. The chemical properties of the soil, biogas residues and maize straw are presented in Table 1. Biogas residues were amended to the soil at an N rate of 150 kg ha^{-1}, *i.e.* 110 mg kg^{-1} dry soil. Maize straw was amended to yield a C dose which was equivalent to the biogas residues: 2.5 g kg^{-1} dry soil. In addition to maize straw, a solution of mineral N as $(NH_4)_2SO_4$ was added to the soil, at an NH_4^+-N rate equivalent to that used in the BGR treatment: 110 mg kg^{-1} dry soil.

One hundred and 50 g air-dried soil was homogeneously mixed with the above-mentioned organic fertilizers and immediately filled into glass pots (250 mL, 12 cm in height). Soil moisture was adjusted to 75% of the water holding capacity with distilled water for all treatments. Pots were then placed in a dark chamber at 19 °C and a relative air humidity of 65%, and remained open during the incubation.

2.2. Analysis of CO_2 fluxes

Gas emission from each pot was measured 15 times over the 21 day monitoring period, using an adapted closed chamber method [22]. Before gas sampling, the incubation chamber was ventilated for 15 min and then air-tight lids were fitted onto each pot. Zero, 20 and 40 min after sealing, the gas inside the pot was sampled using a gas-tight syringe, and stored in pre-evacuated Exetainer glass bottles (Labco, High Wycombe, UK). CO_2 concentrations were analysed by ECD gas chromatography (Varian Star, 3400 CX, USA). Hourly CO_2 emission rates were calculated from the linear regression of CO_2 concentration versus time. Cumulative CO_2 emissions were estimated by linear interpolation of hourly CO_2 emission rates.

2.3. Kinetics of substrate-induced respiration

The kinetics of substrate-induced growth response (SIGR) in the soil [23] was analysed at the end of intensive decomposition period, 300 h after incubation, using a model presented by Panikov and Sizova [24]. This approach enables estimation of the kinetic parameters of soil microbial growth in response to substrate amendments. It is necessary to stress that all kinetic parameters (specific growth rate, active and total microbial biomass and their turnover times, see below) are reported in reference to the zero time point (before glucose and nutrient addition) and represent the characteristics of the microbial community at that moment. Samples of 10 g soil (on the basis of dry weight) were amended with a substrate mixture containing 10 g kg^{-1} glucose, 20 g kg^{-1} talcum, 1.9 g kg^{-1} $(NH_4)_2SO_4$, 2.25 g kg^{-1} K_2HPO_4 and 3.8 g kg^{-1} $MgSO_4 \cdot 7H_2O$. Soil samples were placed (in triplicate) in an ADC2250 24-channel Soil Respiration System (ADC Bioscientific, Herts, UK) at 19 °C. Each sample was continuously aerated (300 mL min^{-1}), and the rate of CO_2 production from each sample was measured every hour using an infrared detector and mass-flow meter [25].

Table 1 – Chemical properties of the soil and organic fertilizers. TOC – content of total organic carbon; TN – content of total nitrogen; DOC – content of dissolved carbon; all contents are based on dry weight.

	TOC (mg g^{-1})	TN (mg g^{-1})	C/N	DOC (mg g^{-1})	DOC/TOC
Soil	15.0	0.98	15	0.012	0.0008
Maize straw	396	9.4	42	33	0.083
Biogas residues	335	65	5.2	6.4	0.019

The kinetics of microbial growth was estimated by fitting the parameters of Eq. (1) to the measured CO_2 evolution rate [24]:

$$CO_2(t) = A + B\exp(\mu \times t) \quad (1)$$

where A is the initial respiration rate uncoupled from ATP generation; B is the initial rate of the growing fraction of total respiration coupled with ATP generation and cell growth, μ is the specific growth rate of soil microorganisms, and t is time. The parameters of Eq. (1) were optimised by minimising the least-square sum using Model Maker-3 software (SB Technology Ltd.). Three replicates of respiration curves were used for each treatment. Fitting was restricted to the initial phase of the curve, which corresponded to unlimited exponential growth.

Other parameters of microbial growth kinetics were calculated from the optimised parameters of the fitted respiration curve (Eq. (1)). r_0, the so-called physiological state index of the microbial biomass (MB) before substrate addition was calculated by Eq. (2).

$$r_0 = \frac{B(1-\lambda)}{A + B(1-\lambda)} \quad (2)$$

where λ is a basic stoichiometric constant, which has an accepted value of 0.9 [24]. Q, the total specific respiration activity, was calculated by Eq. (3). Y_{CO_2} in Eq. (3) is the MB yield per unit of glucose-C, which was assumed to be constant, with a mean value of 0.6 [26] throughout the monitoring period.

$$Q = \frac{\mu}{\lambda Y_{CO_2}} \quad (3)$$

The duration of the lag period (T_{lag}) was calculated by Eq. (4).

$$T_{lag} = \frac{\ln(A/B)}{\mu} \quad (4)$$

The total microbial biomass (TMB) and active (growing) microbial biomass (AMB) before substrate addition were calculated using Eqs. (5) and (6), respectively.

$$TMB = \frac{B}{r_0 Q} \quad (5)$$

$$AMB = TMB \cdot r_0 \quad (6)$$

Generation times for active and total MB were calculated using Eqs. (7) and (8).

$$T_{AMB} = 1/\mu \quad (7)$$

$$T_{TMB} = T_{TMB}/r_0 \quad (8)$$

A detailed description of the approach used in the present study, and the calculation of all parameters is given by Blagodatskaya et al. [27].

2.4. Measurement of soil enzyme activity

Extracellular enzyme activities in the soil were measured at the end of the incubation period using fluorogenically labelled substrates, according to a modified technique described in Dorodnikov et al. [28]. Five types of artificial fluorogenic substrates were used: 4-methylumbelliferyl-β-D-glucopyranoside (MUF-G, EC 3.2.1.21), for the detection of β-glucosidase activity; 4-methylumbelliferyl-β-D-cellobioside (MUF-C, EC 3.2.1), for the detection of cellobiohydrolase activity; 4-methylumbelliferyl-β-D-xylopyranoside (MUF-X, EC 3.2.1), for the detection of xylanase activity; and 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide dehydrate (MUF-NAG, EC 3.2.1.14), for the detection of chitinase activity. L-Leucine-7-amino-4-methyl coumarin (AMC) was used to study leucine amino peptidase (LAP) activity, which is involved in the hydrolysis of L-peptide bonds. All substrates were purchased from Fluka (Germany).

MUF-substrates were pre-dissolved in 2 mL of 2-methoxyethanol and diluted with sterile distilled water to the desired concentrations. One gram fresh soil sample was extracted with 20 mL water in an overhead shaker (8.33 Hz) for 15 min at room temperature. After shaking, 1 mL of the soil suspension was taken and added to 3 mL prepared MUF-substrate solution, which had been pipetted into a deep well plate (24 × 10 mL wells, HJ-Bioanalytik GmbH, Germany). The plate was incubated at 19 °C for 60 min for enzymes which release monomer units from polymeric chains (β-glucosidase, chitinase and LAP), and for 3 h for enzymes which release cellobiose and xylan (β-cellobiosidase, xylanase). The optimal incubation time for each enzyme was determined in preliminary experiments. Plates were then centrifuged at 196.2 m s^{-2} (20 g) for 5 min. Thereafter, 0.5 mL supernatant was pipetted into 24-well microplates (Becton Dickinson, Franklin Lakes, USA) containing glycine–NaOH buffer solution (pH = 11) to stop the enzyme reactions. Fluorescence was measured at an excitation wavelength of 355 nm, an emission wavelength of 460 nm, and a slit width of 25 nm, using a Victor^3 1420–050 Multilabel Counter (PerkinElmer, Waltham, USA). MUF or AMC calibration solutions were prepared with soil suspensions for concentrations from 0 to 100 nmol. Calibration curves and solvent background were used in every series of enzyme measurements. Enzyme activities were expressed as the MUF or AMC released in units of nanomolar MUF/AMC per gram dried soil per hour (nmol g^{-1} h^{-1}).

2.5. Statistical analysis

The means of three replicates with standard errors are presented. Tukey's HSD post-hoc test was used to identify statistically significant differences among the three treatments at $P < 0.05$. Statistical analyses were conducted using SPSS version 11.5 (SPSS Inc., Chicago, USA). Microbial growth kinetic parameters were fit by non-linear regression of respiration curves (Eq. (1)), using Model Maker-3 software (SB Technology Ltd.).

3. Results

3.1. Cumulative CO_2 emissions

CO_2 emission in the treatments BGR and MST increased significantly ($P < 0.05$) versus the Control soil during the first 48 h (Fig. 1). After 48 h, there was only a small increase in CO_2 emission from BGR-treated soil, whereas CO_2 emission from MST-treated soil continued at a high rate for several days (Fig. 1).

A total of 0.08 g kg^{-1} CO_2 was released from the Control soil over the 21 day incubation period. CO_2 release following MST and BGR treatment corresponded to 0.84 g kg^{-1} and 0.24 g kg^{-1} (Fig. 1). Thus, the cumulative CO_2 emission from soil amended with MST was approximately 2.5 times higher than that amended with BGR. Based on the difference method, (*i.e.* subtraction of the CO_2 emission from the Control soil, without taking the priming effect into consideration), the total CO_2-C emission associated with MST decomposition was 0.76 g kg^{-1}, nearly 30% of the initial C input from the maize straw. In contrast, only 6.4% of the initial C input was mineralised and lost as CO_2 in BGR-treated soil over the 21 day incubation.

3.2. Kinetics of substrate-induced growth respiration

The application of glucose with nutrients resulted in an exponential increase in the CO_2 evolution rate (Fig. 2), indicating microbial growth in all treatments. Compared with Control soil, the substrate-induced respiration rate increased earlier in MST-treated soil, whereas the rate increase was delayed and sloped more gently in BGR-treated soil.

Specific microbial growth rates (μ) in BGR-treated soil was significantly less than those observed for Control and MST-treated soils, indicating the dominance of slower-growing microorganisms in soil treated with biogas residues (Table 2). MST treatment gave a lower value of specific growth rate than Control (Table 2). Both MST and BGR additions increased total MB. The total and growing biomass following

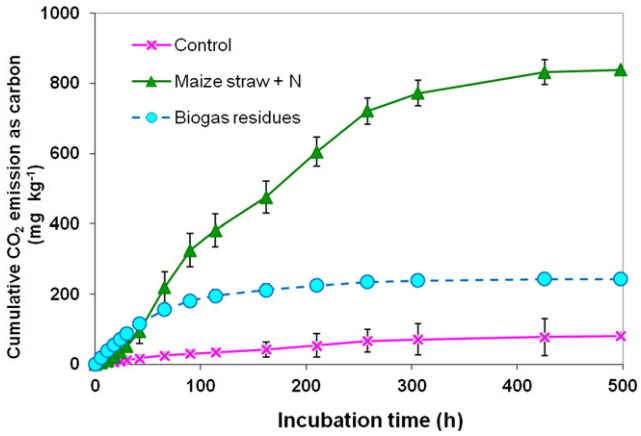

Fig. 1 – Cumulative CO_2 production after addition of biogas residue, maize straw + mineral N, and water (Control). Data are means of 3 replicates ± standard errors. In some cases, error bars are smaller than the symbols.

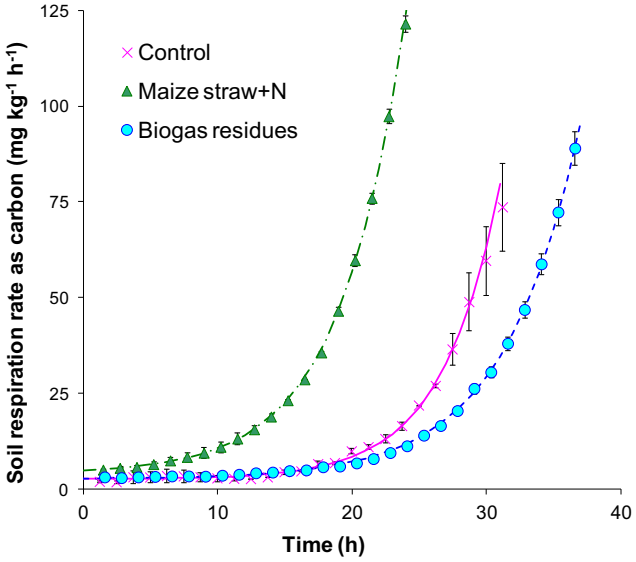

Fig. 2 – Substrate-induced respiration of soil microorganisms in MST, maize straw + mineral N-treated soils; BGR, biogas residues treated soils; Control, soils without amendment. Experimental data are shown as symbols and model simulation as curves.

MST treatment was 82% and 18.5 times higher, respectively, than that observed for the Control, while the percentage of growing biomass in the total biomass increased 10-fold (Table 2). In contrast, BGR was associated with only moderate stimulation of MB: total and active biomass in BGR-treated soil was only 36% and 2.5 times higher, respectively, than in the Control soil. The increase in active biomass mediated by MST was 7.4-fold greater than that observed for BGR.

The generation time of total MB in the treatments MST and BGR was shortened compared with Control (Table 2), and the generation time in the treatment BGR was five times longer than the MST. However, the generation time of active MB was longest in BGR-treated soils (5.6 h); 16% longer than MST-treated soil and 30% longer than Control soil, respectively (Table 2). No significant changes in lag-time (T_{lag}) duration were found between Control and BGR-treated soils. In contrast, the T_{lag} for MST-treated soil was 50% less (ca. 9 h shorter) than the lag-time for Control soil (Table 2).

3.3. *Extracellular enzyme activities*

Extracellular β-glucosidase displayed the highest activity compared to other enzymes in all three treatments, ranging from 1.34 to 1.94 μmol g^{-1} h^{-1}, whereas xylanase activity was the lowest; ca. 20 times lower than that of β-glucosidase (Fig. 3). MST addition increased the activities of all measured extracellular enzymes compared to the Control, with β-glucosidase, cellobiohydrolase, xylanase and chitinase activities increasing significantly (LAP activity was also higher but this increase was not statistically significant). In contrast, treatment with BGR had a differential impact on the activities of C-cycle versus N-cycle related enzymes. Specifically, the activities of N-cycle related enzymes (chitinase and LAP)

Table 2 – Microbial characteristics of growth response, lag-time, actively growing biomass, total microbial biomass and their generation times, calculated by the kinetic approach.

Treatments	Specific growth rate, μ (h^{-1})	Initial non-growth respiration, A CO_2 – carbon (mg kg^{-1})	Initial growth respiration, B CO_2 – carbon (mg kg^{-1})	Lag-time (h)	Microbial biomass			Generation time	
					Total carbon (mg kg^{-1})	Active carbon (mg kg^{-1})	Active (% of total)	Total biomass (d)	Active biomass (h)
Control	0.235 ± 0.013b	2.53 ± 0.49ab	0.053 ± 0.03a	16.5	107.1	0.2	0.21	84.7	4.3
MST	0.208 ± 0.003b	4.01 ± 0.41b	0.82 ± 0.036c	7.6	194.8	3.9	2.00	10.0	4.8
BGR	0.179 ± 0.001a	2.61 ± 0.12a	0.12 ± 0.01b	17.0	145.4	0.7	0.48	49.1	5.6

MST, soils with addition of maize straw + mineral N; BGR, soils with addition of biogas residues; μ, A and B are simulated values ± standard errors, which were given by Model Maker software. Lag-time, total and growing microbial biomass, generation times of total and growing microbial biomass are calculated according to Eqs. (2)–(4), (7), (8), respectively. The different letters in the same column show mean values which have no overlapping in 95% confidence intervals after paired comparison with control and between treatments.

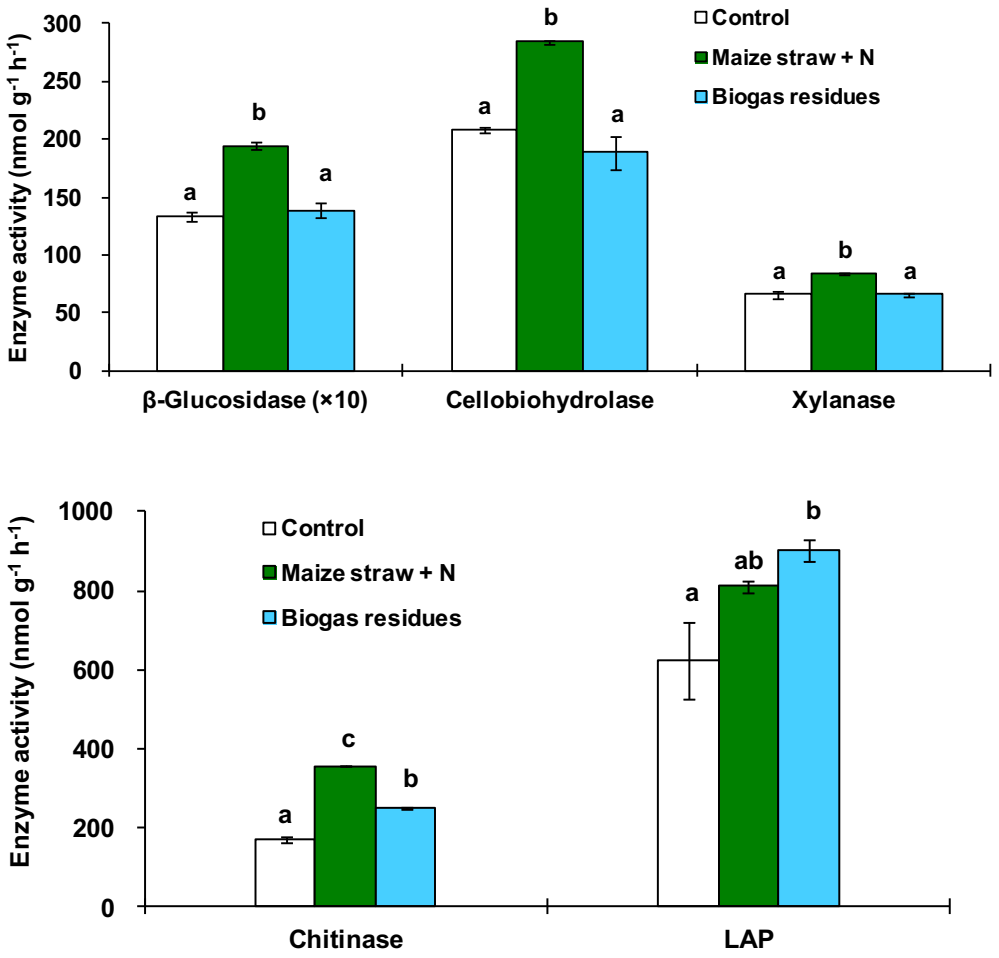

Fig. 3 – Extracellular enzyme activity as influenced by the addition of biogas residue, maize straw + mineral N. C-cycle related enzymes β-glucosidase, cellobiohydrolase and xylanase are at the top and N-cycle related enzymes chitinase and leucine amino peptidase (LAP) are at the bottom. Note, that the values of β-glucosidase activity were divided by ten in order to fit the scale. Data are means of 3 replicates ± standard errors. Letters shows significant differences between the treatments ($P < 0.05$).

significantly increased after BGR addition, whereas no significant changes were observed in the activities of three C-cycle related enzymes (β-glucosidase, cellobiohydrolase and xylanase). In addition, the activities of extracellular enzymes in BGR-treated soil were significantly lower than in MST-treated soil (with the exception of LAP activity, which was highest after BGR amendment).

4. Discussion

4.1. *Decomposition of biogas residues after application*

The cumulative amount of C mineralized in 30 days in soils treated with maize residues was at the upper range of 27–30% of C input reported for different soils [29]. The maize straw is one of the low decomposable plant residues as compared with the residues of soybean (33–38%); sorghum (52–54%) and alfalfa (55–58%) [29]. Five times less amount of CO_2 evolved during the decomposition of BGRs as compared with maize residues in our experiment indicates high resistance of BGRs to microbial degradation. This is explained by lower contribution of cellulose (15 versus 22%), hemicelluloses (20 versus 32%) and higher contribution of lignin (10.5 versus 4.7%) in BGR as compared with maize straw [10,29,30]. Thus, high potential of BGRs in the mitigation of the greenhouse effect when being used as organic fertilizers can be postulated.

The increased SOM content was estimated by the difference between C input and cumulative C mineralization over 21 days, considering that intensive mineralisation of plant residues was completed in 2 weeks [31]. Accordingly, the increase in SOM content caused by applying organic fertilizers was 34% higher in BGR-treated soil than in MST-treated soil. Therefore, land application of BGRs had short-term benefits in terms of improving SOM stock, as compared to common plant residues.

4.2. *Effects of biogas residues on microbial biomass and its growth kinetics*

As expected, application of BGRs and maize straw resulted in an increase in soil microbial biomass (Table 2). The rapid flush

in microbial biomass after incorporation of residues was also reported by other studies [21,32]. Much smaller augmentation of MB caused by BGRs than by maize straw is in agreement with the report of Ernst et al. [33].

Values of μ denote the maximal specific growth rate of microorganisms, which are currently active in the soil sample [23]. As an inherent property of microorganisms, μ can reflect the functional structure of soil microbial community as a whole [27]. Since μ value in BGR-treated soil was significantly lower than in Control soil (Table 1), there was a clear shift in the structure of microbial community in response to BGRs application. The slow-growing microorganisms were dominant among active MB in BGR-treated soil. Such shift in functional structure seems to be related with the changes in fungal-to-bacterial ratio, which responded sensitively to organic fertilization [34], while the genetic structure of soil bacterial community was reported to resist changes within 8 year field experiment [35]. According to our results, a decrease in μ in BGR-treated soil was observed in parallel with increased AMB which is a direct measurement of the physiological state of the microorganisms [24]. Thus, clear increase in activity of K-strategists features reflected the advantage of slow-growing microorganisms in competition for BGRs decomposition. So, application of low available BGRs caused the transition of slow-growing microorganisms from a dormant to an active state and increased their biomass concurrently. The reverse transition in domination from K- to r-strategists was observed after soil amendment with easily available glucose [36] demonstrating that the shift in microbial community composition depends on the availability of applied substrate.

The shortened generation time of TMB (Table 2) in both treatments versus Control indicated a faster turnover rate of TMB caused by fresh substrate input. This turnover acceleration, however, was remarkably lower in BGR- versus MST-treated soil. The extended generation time of AMB reflected a changed structure of active microbial communities upon treatments, and that the population of active microorganisms became dominated by slower-growing species, which has been also demonstrated by μ value.

4.3. *Effects of biogas residues on extracellular enzyme activities*

Our study revealed that the activities of three tested enzymes, β-glucosidase and cellobiohydrolase involved in cellulose decomposition, and xylanase involved in hemicellulose decomposition, were significantly stimulated by MST application, whereas were not affected by BGRs. The MST induced enzymes activation is in agreement with strong raise of the activities of β-glucosidase, phosphatise, protease and xylanase during wheat straw and leaf-litter decomposition [15,37]. The contrasting effects of treatments BGR and MST on the activity of these enzymes might be attributed to the lower C availability in biogas residues versus maize straw. High lignin content in BGRs indicated low C availability [21] due to formation of ligno-cellulose or lignin-polysaccharide complexes [38], which may resist the attack of enzymes.

BGRs application had no effect on activities of β-glucosidase, cellobiohydrolase and xylanase, but significantly promoted chitinase and LAP activities (Fig. 2). The activity of the latter two enzymes is related to N-cycle [39] and is promoted by N-enriched organic components, e.g. peptidoglucan accumulated as microbial residues during the biogas fermentation [40]. Since chitin is mainly derived from fungal cell walls in soils, the increased chitinase activity (Fig. 2) implied a stimulation of fungi in BGR-treated soil, and a faster turnover of fungi compared with Control due to the decomposition of dead fungal mycelium through microbial succession. In BGR treatment lower growth rates were accompanied by increase in chitinase activity. As fungi usually demonstrate slower growth as compared with bacteria [41] we assume the shift to fungal dominance in BGR-treated soil. Increased proportion of fungal biomass associated with surface residue amendments [42] was explained by the benefiting ability of fungi over bacteria to utilize C from residues and N from the soil [31]. Despite mineral N was added equally in our experiment, the stimulation of chitinase was stronger in MST than in BGR treatment. Larger shortage of N due to higher amount and a faster turnover of TMB was considered to be a possible reason of increased chitinase activity in MST versus BGR treatment.

4.4. *Soil C turnover and microbial features after BGRs application*

Although the total organic C input was equal for the BGR and MST treatments, the carbon loss from the soil due to the mineralization were significantly lower in BGR than in MST treatment illustrating that decomposition rate of added organic materials is mainly dependent on its composition and availability [31]. The DOC content and DOC/TOC ratio of biogas residues was only 1/5 that of the maize straw (Table 1), indicating that maize straw had a higher availability of the labile C than biogas residues. This was due to 30–40% larger cellulose and hemicelluloses content and twice less lignin content in maize straw versus BGRs [30,43].

Soil amendments with organic material which cannot be rapidly mineralized by microorganisms and do not cause a strong acceleration in microbial activity are preferable to improve the C stock [44]. The large increase in microbial biomass and high activity of hydrolytic enzymes in MST treatment (Table 2) reflect the proliferation of soil microorganisms caused by labile C input [45]. Compared to MST treatment, the longer lag-time, moderate activation of soil microorganisms and no changes in activities of C-cycle related enzymes (Fig. 2 and Table 2) were observed in BGR-treated soil. This can be attributed to the lower degradability of BGRs compared to MST [46]. Thus, more C remained in BGR- than in MST-treated soil is expected due to slower turnover of microbial biomass C (Table 2) and due to slower mineralisation of BGR-C during incubation [47].

In order to maintain soil SOM content, an organic amendment is requested to diminish a real positive priming effect (PE). Whether the residues produced through anaerobic fermentation induce larger or smaller PE than untreated plant residues [48], animal slurry [49], and green manure [50] remains unclear. Based on the hypothesis that real positive PE is a result of succession of fast- to slow-growing microorganisms [51], both MST and BGR treatments may lead to real PE due to the

clear shift in the microbial community as an evident decrease in μ values (Table 1). However, MST treatment is expected to cause a larger real PE than BGR, as AMB in MST treatment was 5 times of that in BGR treatment (Table 2). The promotion of β-glucosidase and cellobiohydrolase activities by MST treatment is another indicator of real positive PE, because these enzymes are also involved in the decomposition of native SOM [52]. In contrast, the occurrence of PE in BGR treatment was not supported with the increase of C-cycle related enzymes.

5. Conclusions

Compared to untreated maize straw, BGRs had lower C availability, and its application resulted in a shift of active microbial community to slower-growing microorganisms, as well as in a moderate increase in microbial biomass. BGRs application did not promote the activity of extracellular enzymes involved in the decomposition of cellulose and hemicelluloses. We conclude that BGRs displayed a lower mineralisation rate than unfermented plant residues, and potentially had a smaller real priming effect on SOM decomposition. The application of BGRs as organic fertilizers may contribute to mitigation of CO_2 emissions to the atmosphere and had short-term benefits in terms of improving SOM stock, as compared to common plant residues. As our study was based on an incubation experiment, more field observations are needed to evaluate fully the applicability of BGRs under natural climatic conditions.

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