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

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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 CO2 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 CO2 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 ( $\mu$ ) 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  $\mu$  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. © 2012 Elsevier Ltd. All rights reserved.

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Abbreviations  BGR biogas residues  T <sub>lag</sub> 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
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#### 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<sub>2</sub> [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 agroecosystems.

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 airdried, 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

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