



# Gasification biochar has limited effects on functional and structural diversity of soil microbial communities in a temperate agroecosystem



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## ABSTRACT

Biochar may enhance soil fertility and carbon (C) sequestration but there is still a lack of comprehensive understanding of its effects on soil microbial communities and functioning. This study tested the differential effects of two doses (6–8 and 0.8–1.4 t ha<sup>-1</sup> for High and Low doses, respectively) of wheat straw gasification biochar (GBC) and fresh straw incorporated as soil amendments into an agricultural field in Denmark. Soils were analysed three months after the amendments for pH, total organic matter, microbial biomass (ATP), ten enzymatic activities, catabolic potential by substrate-induced respiration (MicroResp™), soil toxicity test (BioTox™) and bacterial community structure (Illumina 16S rRNA gene sequencing). No significant effect of biochar treatment was observed regarding ATP content, catabolic community profiles and soil toxicity. The higher dose of GBC increased phenol oxidase activity and soil pH, and decreased the cellulase activity. No major effect of high dose GBC was observed on the soil community diversity, and only minor effect on the community composition, with an increase in the relative abundance of a single OTU associated with *Acidobacteria\_Gp16*. Addition of low dose of GBC caused an increase in the relative abundance of the rare members in the microbial communities thus increasing the diversity of soil microorganisms. A comparable effect was observed with the addition of fresh straw. Overall, our results indicated that GBC as soil amendment had a limited effect on the functional and structural diversity of soil microbial communities in a Danish temperate agroecosystem.

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## 1. Introduction

Incorporation of charred plant residues into agricultural soils has multiple positive effects on the environment by obtaining renewable energy during biochar production, improving the utilisation of a variety of low-value and challenging biomass fuels from different sectors of society, returning nutrients to the field, and thus closing the nutrient loops in agroecosystems (Lehmann, 2007). Biochar is, by definition, charred organic matter that can

be applied to soil in a deliberate manner with the intention of improving soil properties (Lehmann and Joseph, 2009). There is a growing interest in biochar application to soil as a strategy to improve fertility and mitigate CO<sub>2</sub> emission to the atmosphere by carbon (C) sequestration (Cernansky, 2015; Lehmann and Joseph, 2009). The effects of charred matters on soil fertility depend on their chemical properties, their impact on the soil microbiota and key soil functions caused by alkalisation, release of trace elements and volatile organic compounds, (VOCs) (Deenik et al., 2010; Graber et al., 2010; Spokas et al., 2011; Sun et al., 2015), and by alteration of the nutrient availability. In biochar-amended soils, nutrient availability depends on level of soil alkalinity that enhances the release of nutrients as dissolved organic C (DOC), nitrogen (N), and phosphorous (P) and their complexation with the reactive functional groups onto the surface of the char particles (Joseph et al., 2015;

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Mukherjee and Zimmerman, 2013; Müller-Stöver et al., 2012). The biochar impact on the soil microbial communities depends on the quality of the biochar in terms of feedstock, pyrolysis and ageing, but also on the site characteristics in terms of soil properties (such as texture, mineralogy, pH and cation exchange capacity) and soil management (Dai et al., 2013; Grossman et al., 2010).

The porosity and high specific surface area of biochar increase the inhabitable space for microorganisms or their adhesion to the surfaces and may support larger microbial biomass through the increase of colonisable or protected niches (Lehmann et al., 2011). Moreover, microbial abundance could be positively influenced by the capacity of biochar to adsorb microbial growth-regulating compounds, nutrients or water (Lehmann et al., 2011).

Recent studies have addressed the effects of biochar on soil microbial communities (Anderson et al., 2011; Chen et al., 2013; Rutigliano et al., 2014; Watzinger et al., 2014), reporting variable effects in terms of soil microbial community structure and richness (Sun et al., 2013). However, due to differences in properties of the biochars associated with feedstock characteristics, production technology and conditions, the effects of biochar on soil functioning and microbial community structure are difficult to generalize (Lehmann et al., 2011).

Low temperature-circulating fluidized bed gasification (LT-CFB) is a promising process for producing electricity and heat with a high efficiency (Ahrenfeldt et al., 2013) while providing a char fraction as a potential product of high fertilizer value, like the wheat straw gasification biochar (GBC) used in the present study. The process is based on separate pyrolysis and gasification with maximum temperatures of 700–750 °C, leaving most of the nutrients of the original feedstock on the GBC particles (Hansen et al., 2015; Müller-Stöver et al., 2012). The LT-CFB technology produces GBC with a low content of polycyclic aromatic hydrocarbons and heavy metals, and with a high proportion of recalcitrant C, which can lead to an effective C sequestration in agricultural soils (Hansen et al., 2015; Müller-Stöver et al., 2012). The use of fresh straw for GBC production and its subsequent use on arable land as renewable soil amendment will reduce the costs and improve the sustainability of farmers' production, while utilizing the beneficial effects of GBC for potentially improved soil functions and services (Galinato et al., 2011). Moreover, due to the extra nutrient pool possibly being released in soil, the use of GBC can increase the efficiency of fertilizer use in agricultural soils due to changes in e.g. soil water retention and root development (Bruun et al., 2014; Hansen et al., 2016).

Based on the physico-chemical properties of GBC produced with the LT-CFB technology, we hypothesized that GBC produced from wheat straw could have beneficial effects on soil microbial biomass, bacterial diversity (increasing size and diversity of soil bacterial communities) and metabolic activity, with no adverse effects on soil biological functions. We also compared the effects of GBC with those of fresh straw incorporation, the typical scenario for arable soil in Denmark. The overall goal of this study was to evaluate if soil amendment with a new type of biochar (GBC) could become a sustainable agricultural practice.

## 2. Materials and methods

### 2.1. Site description and treatments

Soil was sampled the 1st of November 2013 from an agricultural field trial at Bregentved Estate, Zealand, Denmark (55° 22' N, 12° 05' E). It was a typical cereal-rich rotation with catch crops grown in autumn before spring cereals and with oil seed rape grown every fourth year (farm director Anders Dolmer pers. comm.). Since 2005, the soil has been under reduced tillage (0–15 cm top soil) in

combination with straw incorporation. All mechanical field operations, mineral fertilizer and pesticide application were performed following Danish national regulations and recommendations and applied according to plant demands (more information can be found at <http://agrifish.dk/>). Soil texture was: 14% clay, 14% silt, 47% fine sand and 24% coarse sand, the total C and N contents were 1.98% and 0.18%, respectively. The GBC used in this study was produced from winter wheat (*Triticum aestivum* L.) by LT-CFB at max temperatures of 700–750 °C. The GBC used had a pH value (in water) of 11.6 and a BET specific surface area of 75 m<sup>2</sup> g<sup>-1</sup>, for more details see Hansen et al. (2015). The P content in the GBC was 0.4% in 2012 and 0.5% in 2013. The Bregentved field trial consisted of three replicates (plots of 0.12 ha each) with four treatments, arranged in fully randomized blocks.

The study was conducted in 2012 and 2013, when winter wheat and oil seed rape were grown, respectively. According to crop needs, all treatments received recommended optimum mineral N fertilizer (192 and 233 kg ha<sup>-1</sup> in 2012 and 2013 respectively), and were replenished with mineral fertilizer (P and K) to meet the annual crop requirements (Table 1).

The four treatments included amendment of two doses of GBC corresponding to the crop P demand (8 and 6 t ha<sup>-1</sup> in 2012 and 2013, respectively; High GBC) and to the K demand (0.8 and 1.4 t ha<sup>-1</sup> in 2012 and 2013, respectively; Low GBC), traditional straw incorporation (Straw), and an unamended soil (Control) (Table 1).

The GBC was added after being moistened with water; using a standard lime and fertilizer spreader (Bredal K105) and incorporated by an airseeder combined with a harrow (Horsch Focus). The straw was incorporated using a harrow (Horsch Joker). All amendments were incorporated into the soil to a depth of 15 cm in September 2012 and August 2013 before crop seeding.

### 2.2. Soil sampling

At the 1st of November 2013 (3 months after the second year incorporation of the amendments), soil was sampled in all plots, during the oil seed rape season. Twelve samples from the three replicates of the four treatments, each consisting of a pool of 10 separated soil cores per each plot, were taken from the Ap horizon (0–15 cm) using a hand auger (2 cm diameter). All samples were homogenized and sieved at field moisture to obtain a fraction ≤4 mm. The samples were air-dried for chemical analyses. For biochemical analyses, samples were stored at 4 °C and for molecular analyses, the samples were stored at –80 °C.

### 2.3. Soil chemical parameters

Soil was analysed for pH, dissolved organic carbon (DOC), mineral nitrogen (N<sub>min</sub>) and total organic carbon (TOC) according to Müller-Stöver et al. (2012). Results were expressed on a dry weight basis (Table 2). The soil organic matter (SOM) content was estimated by loss on ignition (LOI) method performed at 550 °C according to Heiri et al. (2001).

Total P was determined on soil treated at 550 °C followed by extraction with 0.5 M sulfuric acid as described by Bowman (1989). The phyto-available P fraction was determined by the Olsen bicarbonate extractable P fraction (Olsen-P) using 2 g of soil and was quantified colorimetrically by an automatic colorimeter (Auto-Analyzer 3, Bran + Luebbe, Norderstedt, Germany) as described by Müller-Stöver et al. (2012).

### 2.4. Soil microbial biomass and toxicity test

Soil microbial biomass was estimated from the adenosine

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