



# Organic matter dynamics, soil aggregation and microbial biomass and activity in Technosols created with metalliferous mine residues, biochar and marble waste

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## ABSTRACT

Creation of Technosols by use of different materials can be a sustainable strategy to reclaim mine tailings spread on the environment. A proper selection of materials is critical to efficiently contribute to soil creation, with development of soil structure, organic matter stabilization and stimulation of microbial growth. For this purpose, a 90 days incubation experiment was designed with biochars derived from different feedstocks, added to tailings alone or in combination with marble waste (MaW). We aimed to assess the effects of the different materials on the evolution of C and N contents and pools, greenhouse gas (GHG) emissions, aggregate stability, and microbial biomass and activity. Results showed that carbonates provided by MaW increased pH around the target value of 8, with significant decrease in salinity by precipitation of soluble salts. Organic C and total N remained stable during the incubation, with high recalcitrant indices. Labile and soluble C and N pools were low in Technosols, with no differences with unamended tailings at the end of incubation. All biochars increased aggregate stability with regard to control by ~40%, with no effect of addition of MaW. Biochars significantly increased microbial biomass C during the first 7 days of incubation; however, from this date, there were no significant differences with unamended tailings. The  $\beta$ -glucosidase activity was below detection limit in all samples, while arylsterase activity increased in biochar-amended samples favored by increases in pH. CO<sub>2</sub> emissions were not significantly affected by any amendment, while N<sub>2</sub>O emissions increased with the addition of biochars with lower recalcitrance. CH<sub>4</sub> emissions decreased in all Technosols receiving biochar. Thus, the combined use of biochar and MaW contributed to soil C sequestration and improved soil structure. However, labile sources of organic compounds would be needed to stimulate microbial populations in the Technosols.

## 1. Introduction

Metalliferous mine areas constitute degraded ecosystems resulting from mineral extraction for long periods of time, giving rise to large areas of derelict land. Environmental transformations related with mining activities entail changes in the morphology of the area owing to the extraction of minerals and dumping of residues, with reduction of vegetation cover and fauna diversity, and changes in soil quality and structure (Sadhu et al., 2012; Zawadzki et al., 2016). Metalliferous mine residues dumped into the environment have numerous restrictions such as extremely low pH, high concentrations of metals and metalloids and extremely low organic matter content, hindering their development

into soils that support rooted plants without human intervention (Martínez-Pagán et al., 2011; Zanuzzi et al., 2009). These materials can also provoke acid mine drainage, a dangerous source of water contamination (Barrie and Hallberg, 2005). Therefore, there is a need to develop strategies to reduce the impact of mining residues spread on mine landscapes to guarantee ecosystem reclamation. One effective solution might be the creation of Technosols (IUSS, 2014) by addition of different materials or amendments, so that microorganisms, vegetation and fauna are able to colonize and grow. Technosols are soils dominated by human-made material, whose properties and pedogenesis are dominated by their technical origin. Thus, their parent material is a material made or exposed by human activity that otherwise would not

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occur at the Earth's surface (IUSS, 2014). For this purpose, the proper selection of materials and amendments to promote the formation of soil aggregates is critical, since ones of the most important processes for pedogenesis are the accumulation and cycling of soil organic matter and the formation of stable aggregates (Huot et al., 2014; Séré et al., 2010). Soil aggregation determines the soil pore network and thus contributes to root stretching, water infiltration and retention, aeration, diffusion of nutrients, movement of fauna and creation of different niches for microorganisms (Atkinson et al., 2009; Wang et al., 2017).

Organic residues are commonly used as amendments because the addition of organic matter can significantly improve soil structure and nutrient status (Senesi et al., 2007). However, if organic matter provided by the amendment is not properly stabilized, it is rapidly degraded and mineralized by soil microorganisms, not contributing to promote soil aggregation and improve soil structure. For example, Zornoza et al. (2013) reported that organic matter provided by pig slurry was rapidly mineralized in soils, with no significant increases in soil organic carbon after some weeks. Biochar production through the pyrolysis of organic residues has recently become an interesting solution for successful soil reclamation due to its high recalcitrant carbon content, which can increase the content of stable organic carbon in soil, contribute to carbon sequestration, and improve soil physical, chemical and biological properties (Glaser et al., 2002; Lehmann and Rondon, 2006; Marchetti et al., 2012). Although biochar is mostly formed by recalcitrant organic compounds, it also contains soluble and organic labile pools and nutrients which can stimulate microbial growth (Zornoza et al., 2016a, 2016b). There is no general formula or standard definition for the recalcitrant, labile or soluble organic fractions. Nonetheless, operationally it refers to resistance to loss under selected chemical treatments (Pandey et al., 2014). Hydrolysis with acids like HCl and H<sub>2</sub>SO<sub>4</sub> (Rovira and Vallejo, 2000) are common procedures to obtain labile and recalcitrant carbon pools, simulating how stable organic compounds are to chemical and biological degradation in soil. Regardless of differences among methods, the simplest fractionation may be to divide organic carbon into three major pools: a soluble pool characterized by mean residence time (MRT) of few weeks, a labile pool characterized by an MRT of years to a few decades, and a recalcitrant pool with MRTs ranging from hundreds to thousands of years (Cheng et al., 2007).

Biochar has also emerged as a strategy for reducing greenhouse gas (GHG) emissions. However, the effect of biochar on GHG highly depends on biochar feedstock and pyrolysis conditions, soil type and soil management (Cayuela et al., 2015; Hüppi et al., 2015; Van Zwieten et al., 2010). Biochar is a highly alkaline material (Paz-Ferreiro et al., 2014), and has also resulted highly effective to increase pH in acidic soils (Ahmad et al., 2014; Moon et al., 2013; Paz-Ferreiro et al., 2014). The importance of calcareous materials in supplying carbonates and Ca ions to neutralize acidity, contributing to buffer capacity and stabilize soil organic matter is well recognized (Zornoza et al., 2013). For these reasons, several authors have suggested the use of marble wastes as a source of calcium carbonate for acidic soil remediation (Janjirawuttikul et al., 2011; Tozzin et al., 2014; Zornoza et al., 2013).

Previous studies have reported the positive effects of Technosols created on mine and industrial residues through the addition of different amendments to ensure true landscape reclamation (Huot et al., 2014; Kelly et al., 2014; Séré et al., 2010; Wiszniewski et al., 2016). However, to really conclude that the addition of different organic and industrial materials to mine residues efficiently contributes to the formation of a healthy soil with presence of stable soil aggregates, soil organic matter and active microbial communities, a proper and thorough monitoring of organic matter stability, GHG emissions, aggregation, and microbial biomass and activity should be developed. Since soil microorganisms are key in the stabilization and degradation of soil organic matter and in the formation of soil stable aggregates (Aegehehu et al., 2016; Zornoza et al., 2016a), the monitoring of microbial biomass and activity, and its interaction with

soil physicochemical properties will help gain knowledge about microbial mediated processes in Technosols. It has been reported that biochar can alter soil microbial community structure and abundance. Increases in the abundance of Gram negative bacteria with biochar addition have been previously observed (Mitchell et al., 2015; Prayogo et al., 2013; Yanardağ et al., 2017). Nonetheless, most studies have reported that *Actinobacteria* are the microbial group with higher growth and abundance after biochar addition to soils (Dai et al., 2017; Sun et al., 2016; Watzinger et al., 2014; Yanardağ et al., 2017). In order to elucidate the main factors controlling organic matter stabilization and degradation and soil aggregates formation in a Technosol derived from mine residues as strategy for reclamation, we performed a short-term incubation experiment with biochars derived from different feedstock and marble waste. The final aim of the Technosols creation is to provide ecosystem services such as C sequestration, biomass production and biodiversity reservoir. The use of organic residues such as pig manure, crop residues or municipal solid waste (used as feedstock for biochar production in this experiment) for soil reclamation has been extensively studied, with positive effects on soil quality and health. However, although there is increasing concern about the potential use of biochar to increase soil organic matter and fertility, little information is still available about its benefits and limitations for Technosols creation. Studies on the use of biochar as organic material for tailings reclamation are needed so that robust scientific results arise to confirm if it is an effective option for land managers. Thus, our objective was to assess the effects of the different materials on the evolution of C and N contents and pools, GHG emissions (CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub>), aggregate stability, and microbial biomass and activity. We hypothesized that the application of biochar and marble waste to mine soils can contribute to the stabilization of organic matter by formation of stable aggregates and the activation of microbial populations. Soil organic matter stabilization is needed to maintain organic C reservoir in soil, with positive effects on soil structure, water retention, aeration, fertility, rooting, fauna development and microbial biomass and diversity. Biochar type could have a strong influence on aggregate stability and microbial stimulation, since feedstock determines organic matter quality (presence of different functional groups) and nutrient content (Paz-Ferreiro et al., 2014; Zornoza et al., 2016b). Addition of MaW may have a positive effect on soil aggregation, since the acidic nature of mine tailings promotes the rapid dissolution of added CaCO<sub>3</sub>. The Ca<sup>2+</sup> released can bridge organic matter and mineral particles resulting in the formation of soil aggregates (Zanuzzi et al., 2009). However, although in forest and agricultural soils biochar addition can enhance and activate microbial populations, in soils derived from mine tailings, with absence of native organic matter, biochar may not be effective owing to its high recalcitrance if no sufficient labile organic compounds are released. If this was the case, with this study, land managers would have support data not to use biochar alone to reclaim tailings.

## 2. Materials and methods

### 2.1. Mine, organic and industrial residues used

A pyritic tailings pond at the Mining District of Cartagena-La Unión (SE Spain) (37°35'38" N, 0°53'11" W) was selected to collect the mine residues, which were characterized by high acidity and metal concentrations, low organic matter and nutrient contents, and sandy loam texture (Table 1). The mine residue was collected from the top 20 cm of the tailings pond, air-dried for seven days, and sieved < 4 mm for incubation experiments, so that coarse fragments were discarded.

Biochar feedstock was pig manure, cotton (*Gossypium hirsutum* L.) crop residues and municipal solid waste, collected in a pig farm, agricultural field and municipal solid waste treatment plant from the municipality of Cartagena (SE Spain). The feedstock was dried at 60 °C in a forced air lab oven during 72 h and then was ground to pass a 4-mm sieve. The ground particles were then pyrolyzed in a muffle

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