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Soil Biology & Biochemistry 39 (2007) 1493–1503

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

A standardized method for the determination of the intrinsic carbon and nitrogen mineralization capacity of natural organic matter sources

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Received 31 May 2006; received in revised form 14 December 2006; accepted 20 December 2006
Available online 8 February 2007

Abstract

A new method was developed for the simultaneous determination of the intrinsic carbon and nitrogen mineralization capacity of organic matter (OM) sources by means of an aerobic incubation in suspension. The proposed method is based on determination of the oxygen consumption, monitored indirectly via pressure measurement, and on determination of nitrogen mineralization, through the periodical measurement of NH_4^+-N, in a liquid suspension of the samples. The suspension is standardized in terms of nutrient composition and pH, and well-controlled incubation conditions that can be enforced as desired. This method rules out the effect of soil conditions and thus reflects the intrinsic properties of the OM. The method is faster and more reproducible than soil incubation tests that are currently used. In such a system, it is important that nitrification is inhibited to avoid oxygen consumption by nitrifiers and prevent the production of gaseous nitrogen compounds. Two nitrification inhibitors, *N*-allylthiourea and 2-ethynylpyridine, were tested at different concentrations for three reference samples, soil, bark and manure. Both inhibitors completely suppress NO_3^- formation without suppressing the heterotrophic microbial activity, thus allowing the correct determination of the oxygen uptake rate (OUR). When nitrification inhibitors were added, nitrous oxide could not be detected anymore in the gas phase of the system, which confirms that nitrification was inhibited and indicates that denitrification and nitrifier denitrification activity was negligible. N mineralization rates were determined by frequent sampling from the liquid phase of the system without disturbing the pressure measurement during the incubation and subsequent determination of NH_4^+-N. The method presented allows for the reliable and relatively easy and cheap, simultaneous determination of carbon and nitrogen mineralization rates for a wide range of OM sources.

Keywords: Respiration rate; Carbon; Nitrogen; Mineralization; Liquid environment incubation; Nitrification inhibitors; Organic matter stability

1. Introduction

Agricultural soils and soilless growing substrates are frequently enriched with organic amendments such as crop residues, manures and composts. These organic matter (OM) inputs can supply plant nutrients, increase natural suppressiveness of the soil against plant pathogens and improve physical and chemical soil characteristics such as cation exchange capacity and waterholding capacity (Cookson et al., 2005; Giusquiani et al., 1995; Veeken et al., 2005). However, OM inputs can also lead to negative effects such as temporal oxygen depletion and denitrification, nitrogen immobilization and plant pathogen stimulation (Yamulki, 2006). To maximize the positive and minimize the negative effects of OM inputs it is important to apply a proper quantity and quality of OM at a proper time.

An important aspect of OM quality is its stability. This stability or degradability is an intrinsic property of OM and is determined by its composition, e.g. its content of organic acids, proteins, humic acids and lignin. For a good OM management by farmers and land managers, quick

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0038-0717/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2006.12.035

and reliable tests are needed that can be used to evaluate quality of OM sources in order to choose the most suitable source for a specific situation. These tests are e.g. needed to develop adequate fertilization strategies that consider not only mineral N levels at the start of a cropping season but also the amount of N that can be expected to become available to plants through mineralization during the growing season and that minimize nutrient losses and environmental damage due to NH_3 volatilization, NO_3^- leaching and N_2O emissions (Chaves et al., 2005; Erhart et al., 2005). Also for the container-grown plant industry these tests are of increasing value as we see an increase in the use of composts to partially replace peat in potting mixes (Evanylo and Daniels, 1999; Abad et al., 2001; Bugbee, 2002; Papafotiou et al., 2004). A careful selection of high-quality composts is needed to avoid phytotoxicity and nitrogen immobilization and to optimize microbial activity such that disease suppression is maximized (Iannotti et al., 1993, 1994; Veeken et al., 2005). For tests that aim at characterizing OM quality, both carbon and nitrogen dynamics are relevant to consider because C and N are the main determinants of OM decomposition rate, N is the plant nutrient that is most difficult to manage because of its many different forms, some of which are easily lost for crop production, and because C and N dynamics are strongly interrelated.

To evaluate stability or relative N supplying capacity of OM sources, different approaches have been used. Many tests are soil incubation tests in which OM is mixed through soil and incubated under standardized conditions (Bernal et al., 1998; Chaves et al., 2004). However, these tests are time and labor intensive, the incubation time is relatively long (2–36 week) and the results are often poorly reproducible. The latter is caused by the fact that results are dependent on characteristics of the specific soil used for testing, such as pH, soil texture, initial OM content and nutrient content, and because some factors are not easily standardized such as soil porosity or kept the same over a longer period of time such as soil moisture content (Jensen et al., 1996; Bernal et al., 1998; Agehara and Warncke, 2005; Beraud et al., 2005; Khalil et al., 2005; McDowell et al., 2006). A well-recognized problem with the soil incubation tests is the risk of development of anaerobic microsites depending on air-filled porosity and OM loadings. In the microsites, denitrification will result in N losses. These losses are rarely taken into account, leading to incorrect estimates for nitrogen mineralization rates (Bernal et al., 1998; Agehara and Warncke, 2005). These drawbacks indicate that soil incubation tests are not well suitable for routine testing and that there is a need for other tests.

Alternative methods determine the O_2 consumption of samples (Iannotti et al., 1993, 1994; Lasaridi and Stentiford, 1996, 1998; USCC, 1997; Adani et al., 2004; Barrena Gómez et al., 2005). In the respirometric tests in which unsaturated samples are incubated, the O_2 transfer to the bacterial cell wall is recognized to be the rate limiting step (Paletsky and Young, 1995). Higher reproducibility can be reached when respiration is determined in a liquid suspension. A liquid environment avoids differences in matric water potential, allows for good mixing of all substances and promotes direct contact between substrate, microbes and O_2, thus resulting in maximum reaction rates (Lasaridi and Stentiford, 1998). In this study, we use this approach and a specific commercially available measuring system, the OxiTop® system (WTW, Wilhelm, Germany) is used to determine simultaneously C and N mineralization potential of OM sources. In the OxiTop® system, the O_2 consumption is indirectly determined by measuring the pressure in the gas phase above the suspension. As CO_2 is trapped and nitrification is inhibited, O_2 consumption can solely be subscribed to C mineralization. Nitrification inhibition is also important to prevent loss of N in the form of nitrous oxide and to facilitate the measurement of N mineralization as only NH_4^+-N has to be monitored.

The aim of this study is to develop a standardized, reliable, quick and relatively cheap procedure that can be used for the routine testing of OM sources in terms of their C and N mineralization potential. To optimize and evaluate the procedure, three different OM sources, soil, bark and manure, representing stable, intermediate and unstable OM, were used, respectively. The study consists of two parts: (i) configuration of an analytical device to determine simultaneously C and N mineralization rates in a liquid medium for a wide range of samples, and (ii) selection of type and concentration of a nitrification inhibitor that completely suppress nitrification without affecting the microbial degradation of OM. The tests used in this study with different OM sources were run for 168 h.

2. Materials and methods

2.1. Reference materials

Soil, uncomposted bark and animal manure were selected to represent sources of OM with different composition and stability. Soil and fresh, solid cow manure were collected from the organic educational and experimental farm Droevendaal of Wageningen University (NL) and bark was purchased commercially. All samples were dried in a forced ventilation oven at 30 °C until constant moisture content was reached. After drying, samples were milled using a 2-mm sieve and mixed well. Fresh, activated sewage sludge was collected from the activated sludge wastewater treatment plant at Bennekom (NL) and this was used as nitrifier inoculum. To determine total solids (TS), samples were dried at 105 °C until constant weight was reached. Volatile solids (VS) were determined by heating the oven-dry sample at 550 °C for 4 h (AOAC, 2000). Total organic carbon was determined by dichromate oxidation at 160 °C and total nitrogen was determined by the Kjeldahl method (AOAC, 2000). The characteristics of the samples are reported in Table 1.