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Quantitative assessment of soil functioning across a representative range of Dutch soils

Alexander V. Semenov [a,b], Michele C. Pereira e Silva [a,*], Joana Falcão Salles [a], Heike Schmitt [c], Jan Dirk van Elsas [a]

[a] Department of Microbial Ecology, Center for Life Sciences, P.O. Box 11103/Postbus 11103, 9700 CC Groningen, The Netherlands
[b] INCOTEC Europe BV, 1601 BL Enkhuizen, The Netherlands
[c] Utrecht University, Institute for Risk Assessment Science (IRAS), Utrecht, The Netherlands

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ABSTRACT

Soil microorganisms are the most important determinants of soil functioning. In order to understand the relevance of stress-induced changes (e.g. as promoted by genetically modified plants), the natural variation (or normal operating range, NOR) of soil function has to be better understood. Quantitative assessment of the NOR, taking into account the relevant and most sensitive microbial groups, may lead to the first quantitative characterization of the NOR of an entire soil system. Thus, the focus of this work was on quantitative measurements of key genes involved in the nitrogen cycle, next to broader taxonomic assessment of the microbial groups, by real-time PCR as well as by PCR–DGGE and potential activities. In total, a robust dataset of more than 2000 measurements was obtained, and a NOR was developed based upon this dataset. The NOR can be considered as a space containing n dimensions, where n is the number of variables measured. When a soil (at field capacity) is not disturbed, all combinations of the variables fall inside the NOR. The distance between an investigated state and the center of the NOR represents a quantitative measurement that summarizes the state of the soil, taking into account the multivariate nature of the data. Parameterization of the model was done using microcosm experiments as well as via sampling of selected field soils during 3-years period. One advantage of the proposed approach is that the data itself show which variables are of concern and contribute most to the NOR, next to which ones produce noise. The method will assist in distinguishing the critical parameters in soil which are outside of the NOR as well as in the prevention of unnecessary actions.

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1. Introduction

Living soils constitute natural resources that must be secured for future generations, as the rates of soil formation or recovery are often too low to cope with losses and degradation (Pulleman et al., 2012). The microorganisms inhabiting soil (bacteria, archaea and fungi) are key drivers of the life support functions (LSF) of the system. For instance, they play key roles in the cycling of carbon (Högberg et al., 2001), nitrogen (Kowalchuk and Stephen, 2001) and sulphur (Deng and Tabatabai, 1997; Schmalenberger et al., 2008), next to assisting in soil formation (Rillig and Mummey, 2006). Between 80 and 90% of the relevant soil functions are mediated by soil microorganisms (Nannipiere et al., 2003). Given these facts,

frameworks for the evaluation and monitoring of the status of soil have been designed (Dominati et al., 2010; Robinson and Lebron, 2010). Due to the complex nature of the soil microbial community, it is not possible to measure and exploit all soil biotic (microorganisms) and abiotic parameters. However, from the range of soil biotic parameters that might be addressed, those that are known to be involved in important and sensitive steps of the biogeochemical cycles in soil have been advocated as good indicators of the status of a soil (Domsch et al., 1983; Kowalchuk et al., 2003; Bruinsma et al., 2003).

A case in point is offered by the many C- and N-related proxies, however such numerous parameters cannot be measured routinely. Therefore, considering the major importance of nitrogen in agricultural soils, we have chosen to place a focus on the key microbial groups involved in the nitrogen cycle, e.g. nitrogen fixers, ammonia oxidizers and denitrifiers, next to a range of broader taxonomic groups (bacteria, archaea and fungi). We place a focus on the N cycle because, from our previous results (Pereira e Silva et al., 2011, 2012a,b, 2013), the genes involved in this cycle turned

out to be of great potential and sensitive enough to catch various changes in soil. However, parameters exemplifying other nutrient cycles are obviously also relevant and offer testable indicator proxies in future experiments. It has been pointed out that a suite of different indicators jointly provides a superior measure of the status of a complex system such as an organism (Depledge, 1990), a population or a soil (Van Straalen, 2003). Moreover, efficient evaluation of indicators of soil quality demands community functional assessment under different levels of environmental changes. In this sense, the use of genetic markers involved in the nitrogen cycle (i.e. nitrogen fixation, nitrification and denitrification) offers a broad array of potential proxies. These processes are differentially affected by specific environmental pressures, and they occur at different levels of phylogenetic distribution in microbial populations in soil.

In natural conditions, the status of a soil is expected to fluctuate without clear direction, as the local environment in which soil organisms dwell is variable, however without direction. Such dynamics can be depicted as a sequential occurrence of maxima and minima in the parameters that define the rate of soil processes (Pereira e Silva et al., 2013). Given our need to assess the status of a soil, particular ranges may be considered to be "normal" for the system; such an assessment should take into account that the normality range might differ in accordance with the intended use of the soil, i.e. whether it is used for sustaining houses or for agricultural purposes. Moreover, the status of a system may be considered as a multivariate property, and a stress or impact can be defined as a deviation from this status. The concept of stress itself can only be defined in comparison with a reference, which can be depicted as a baseline state in multidimensional space (Van Straalen, 2002). Assessments of variation in important and sensitive microbial groups may lead to the first – to the best of our knowledge – quantitative characterization of the normal operating range (NOR) of a soil.

In this context, Kersting (1984) defined a 95% confidence space of undisturbed states as the NOR of a system, when evaluating an aquatic microcosm experiment. In this study, the system was stressed by introducing pesticides. The approach allowed for the identification of stress wherever the selected state variables fell outside of the NOR. The definition of a standard of functioning is instrumental in ecotoxicology, where pollutants are considered to represent stressors of microbial systems if the measured state variables fall outside the NOR or if a shift in the NOR is observed (Medina et al., 2007; Schmitt-Jansen and Altenburger, 2005). Nevertheless, a strict definition of the NOR has not been developed for living soils. For instance, we do not know how the magnitude of the NOR may vary according to the soil studied.

In order to evaluate the effect of management (or stress-induced impacts) on soil, a better understanding of the variations in soil functioning caused by natural effects are needed. In this sense, the use of mathematical treatment of soil metadata is a sophisticated way to address this. Up to now, only few mathematical methods addressed the NOR of a soil (Dominati et al., 2010; Rutgers et al., 2012; Van Wijnen et al., 2012). Such methods were based on a combination of expert opinions and classical soil parameters, such as total carbon and/or nitrogen levels, however they did not consider recent investigations of soil functioning (Pereira e Silva et al., 2012a,b). Here, we describe an approach that was evaluated as optimal in terms of the quality of results obtained, accuracy and possible applicability by end users. Our approach takes into account a range of soil and microbial measurements simultaneously. Parameterization was done by using data from microcosm experiments as well as from selected field soils. The study also aimed to distinguish the key soil parameters that influence the NOR, allowing to define a stress-explanatory NOR.

2. Materials and methods

2.1. Data set description for the establishment of a soil NOR

Eight soil sites were sampled across the Netherlands. These include four sandy (B, V, D, W) and four clay soils (S, K, G, L). Sampling points were selected to reflect spatial and temporal variability in external parameters, with replicate bulk soil samples collected in a composite scheme four times over an annual cycle in 2009 (April, June, September and November), three times in 2010 (April, June and October), and four times in 2011 (February, April, July and September). Their chemical and microbiological characteristics, geographical coordinates as well as sampling procedure are found in Tables S1 and S2. More details on site characteristics were previously described (Pereira e Silva et al., 2011, 2012a).

For the development of the model, data on measurable key processes, including disturbance-sensitive ones, were used (Pereira e Silva et al., 2011, 2012a,b) (Table 1). The model was validated and tested with two sets of experimental data, from field experiments (Pereira e Silva et al., 2012a,b; Salles et al., unpublished) and from a microcosm stress experiment performed in this study. Moreover, to validate the importance of N-related variables in comparison with the full set of general variables, soil samples collected in the island of Schiermonnikoog (The Netherlands) were used as a stressed soil, as it is characterized by constant flooding (tide), in addition of the high variability in soil temperature (Schrama et al., 2012), which constitutes a harsh condition for soil microbial community.

2.2. Assessment of disturbances

In order to characterize short-term variations and the influence of stress factors on the microbial communities in soil, a microcosm experiment was carried out. Two different stress conditions were simulated in triplicates. Soil B, presenting sandy texture, was chosen from the eight soil studied in Pereira e Silva et al. (2012a,b). Stress A consisted of subjecting the soil to 30 °C for 12 h followed by addition of water to 100% water holding capacity (WHC) for 12 h and drying it to the initial 65% WHC. Stress B consisted of keeping the soil at 60 °C for 12 h, and then adding water to 100% WHC for 12 h and drying till the initial 65% WHC. Five parameters were selected and measured after the application of the stress, i.e. soil pH, organic matter content, counts of copiotrophic and oligotrophic bacteria and total bacterial diversity based on molecular analyses of the 16S rRNA gene.

2.3. Chemical, biological and molecular characterization of soil microcosms after the application of stress

Soil pH was defined in 0.01 M $CaCl_2$ (1:4.5). Organic matter (OM) content is determined after 4 h at 550 °C. Numbers of copiotrophic and oligotrophic bacteria were quantified by counting CFU on C-rich and C-poor media, respectively. Bacterial colonies were counted after incubation for 60 h on high-C medium (for copiotrophic bacteria) and 14 days on low-C medium (for oligotrophic bacteria). Detailed descriptions of these methods are provided in Franz et al. (2008). Denaturing gradient gel electrophoresis (DGGE) analysis was conducted on total DNA extracted from soil microcosms. The DNA was extracted from 0.5 g of soil using Power Soil MoBio kit (Mo Bio Laboratories Inc., NY), according to the manufacturer's instructions. For DGGE analysis, bacterial 16S rRNA gene was PCR amplified using the forward primer F968 (Gomes et al., 2001) with a GC-clamp attached to 5′ and the universal primer R1401.1b (Brons and Elsas, 2008). PCR cycling conditions are described in Table S3 and details are in Pereira e Silva et al. (2012b). The bacterial diversity was estimated as the Shannon–Wiener index of bacterial diversity (H) (Eichner et al., 1999), which is a diversity index that