Ecological Indicators 93 (2018) 490–500

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Original Articles

Indicators of arable soils fatigue – Bacterial families and genera: A metagenomic approach

Agnieszka Wolińska[a,*], Agnieszka Kuźniar[a], Urszula Zielenkiewicz[b], Artur Banach[a], Mieczysław Błaszczyk[c]

[a] The John Paul II Catholic University of Lublin, Department of Biochemistry and Environmental Chemistry, 1 I Konstantynów St., 20-708 Lublin, Poland
[b] Institute of Biochemistry and Biophysics PAS, Department of Microbial Biochemistry, 5a Pawińskiego St., 02-206 Warsaw, Poland
[c] Warsaw University of Life Sciences, Department of Microbial Biology, 159 Nowoursynowska St., 02-776 Warsaw, Poland

ARTICLE INFO

Keywords:
Soil fatigue
Arable soils
Next generation sequencing
Biodiversity
Soil indicators
Bacterial families
Bacterial genera

ABSTRACT

The main goal of the study was to determine new metagenomic indicators demonstrating sensitivity to soil fatigue as an effect of long-term agricultural use as well as new metagenomic indicators demonstrating bacterial resistance to human agricultural activity. Thirty-one soil samples (agriculturally exploited soil and wastelands – serving as controls) were taken for the study, in the south-eastern part of Poland. For determination of biodiversity, next generation sequencing of the 16S rRNA metagenomic amplicons were used with the Ion Torrent™ technology. Bacterial sequences were clustered into operational taxonomic units (OTUs) based on a 99% similarity threshold. The correlation matrix was constructed to assess the relationships between bacterial families and genera and the environmental data.

In the studied soils, 118 families and 305 genera were identified. Among them, 10 families were recommended as sensitive indicators of soil fatigue: *Sphingomonadaceae* > *Chitinophagaceae* > *Flavobacteriaceae* > *Oxalobacteraceae* > *Acetobacteraceae* > *Myxococcaceae* > *Comamonadaceae* > *Pseudomonadaceae* > *Burkholderiaceae* > *Rhodanobacteraceae*. Analogically, 8 bacterial genera sensitive to agricultural practices were found: *Pelomonas* > *Ramlibacter* > *Flavobacterium* > *Rhizobacter* > *Steroidobacter* > *Cellvibrio* > *Halliangium* > *Pseudomonas*. Their sensitivity was confirmed by a decrease in the number of OTUs in the agricultural soils, in comparison to the wastelands. In contrast, 5 bacterial genera that should be considered as indicators of resistance to agricultural land use were proposed: *Nitrosospira* > *Rhodanobacter* > *Aquicella* > *Burkholderia* > *Mucilaginibacter*. Statistical tests demonstrated that the most important factors for the abundances of bacterial families and genera included soil acidity (pH), easily degradable carbon (EDC), total carbon (TC), nitrite nitrogen ($N\text{-}NO_2$), magnesium (Mg) and calcium (Ca) content, and soil moisture.

1. Introduction

Soil fatigue is often defined as exhaustion of the soil through depletion of nutrients essential for plant growth (Volosciuc and Josu, 2014). Equally often, the aspect of soil fatigue has been analyzed in the physical (da Fonseca et al., 2013), chemical (Guerrero et al., 2014), and biological aspects in relation to emergence of pathogens (Volosciuc and Josu, 2014; Larregla et al., 2015) and overall decline in biodiversity (Wolińska et al., 2015, 2017a,b,c). However, one of the reasons that cause a decline in species diversity and is responsible for soil fatigue is also the agricultural use of soils, evident particularly in areas referred to as poorly managed agricultural sites, and caused by the following factors: ploughing, monoculture, no crop rotation, the use of pesticides, and ploughing plant residues, which cause acidification of the ploughed soil environment (Hobbs et al., 2008; Lal, 2015; Roper et al., 2017; You et al., 2017). It should be emphasized that ploughing mainly causes modifications in the redox potential and water content, resulting in changes in oxygen relations and loss of many species of anaerobic bacteria, because anaerobic zones are destroyed (Zhou et al., 2014; Busari et al., 2015). Therefore, non-agricultural soil is characterized by ca. five-fold greater abundance of anaerobic than aerobic bacteria (Tamames et al., 2010; Rinke et al., 2013). The soil fatigue phenomenon may also be demonstrated by lower biological activity as an effect of human agricultural practices (Wolińska et al., 2017b, 2015, 2016a).

An impact of different land use systems on soil biological and microbiological properties was the subject of many earlier studies (Kibblewhite et al., 2008; Singh et al., 2014; Wolińska et al., 2015; Nugis et al., 2016; Trivedi et al., 2016; Vinhal-Freitas et al., 2017). It is

* Corresponding author.
E-mail address: awolin@kul.pl (A. Wolińska).

https://doi.org/10.1016/j.ecolind.2018.05.033
Received 4 January 2018; Received in revised form 20 April 2018; Accepted 15 May 2018

also commonly known that agricultural soils, especially the long-cultivated ones, may not contain stable microbial communities. Furthermore, the reduction in the number of bacterial phylogenetic groups affected by human agricultural activities is the cause of the low content of taxonomic groups in arable soils (Pershina et al., 2015; Bender et al., 2016; Wolińska et al., 2017a; Zhang et al., 2017). Finally, the lack of biodiversity is another reason for agricultural soils not being able to become naturally regenerated, which may lead to their inability of regaining the satisfactory level of fertility (Goenster et al., 2017). Therefore, long-term cultivated soils may be considered as fatigued (and less fertilized) and it is important to find sensitive and adequate indicators confirming this phenomenon. Guillaume et al. (2016) suggested that direct measurements of soil fertility are impossible because e.g. crop yield reflects only one of many soil services. Hence, soil fertility is usually assessed by determination of factors considered as fertility indicators, e.g. soil organic carbon, microbial biomass, basal respiration, and microbial and enzymatic activities (Askari and Holden, 2014; Dilly et al., 2018).

Until recently, inadequate methods, indirectly connected with the cultivability of microorganisms on artificial media under laboratory conditions, have built the largest barrier for microbiologists, as most bacteria are uncultivable. The latest approaches to estimation of microbial diversity use direct shotgun next generation metagenomic sequencing, which provides effective characterization of the genetic diversity present in samples, regardless of the availability of laboratory culturing techniques (Handelsman, 2004; Chang et al., 2017). Now, via metagenomic tools, it is possible to acquire information about an enormous bacterial group called viable but not cultivable (VBNC), typically comprising 97–99% of soil bacteria (Epstein, 2013; Pershina et al., 2015).

It has also been emphasized that soil microbial indicators are more specific and more sensitive to changes in the land use than biochemical, biological, and physicochemical parameters (Guillaume et al., 2016; Goenster et al., 2017; Vinhal-Freitas et al., 2017). Dilly et al. (2018) indicated that abiotic indicators (i.e. soil texture, bulk density, organic carbon, and nitrogen content) responded to geology and climatic conditions more evidently, whereas microbiological indicators reacted more specifically to soil management practices and land cover. Paradoxically, chemical and physical indicators are determined more frequently than microbiological ones, probably due to their less complicated analytical procedure and wider understanding thereof (Dilly et al., 2018). In contrast, microbiological factors are usually considered to be more difficult for determination and demanded more careful interpretation (Dilly et al., 2018).

Via metagenomic analyses, Wolińska et al. (2017c) determined that, at the taxonomic level, the bacterial phylum *Bacteroidetes* are suitable to be recommended as sensitive indicators of agricultural soil usage, as the number of their operational taxonomic units (OTUs) was significantly reduced in arable soils versus non-cultivated (control) sites. Among *Bacteroidetes*, *Flavobacterium* was found to be the most sensitive bacterial genus to agricultural practices (Wolińska et al., 2017c). Zhang et al. (2017) noted that the abundance of *Firmicutes* and *Actinobacteria* decreased in degraded soils, in comparison to healthy ones. The study conducted by Trivedi et al. (2016) demonstrated that the relative abundance of *Acidobacteria*, *Proteobacteria*, *Actinobacteria*, and *Cyanobacteria* was significantly higher in non-cultivated soils than in agricultural sites. Similarly, it has been found that potential nitrogen-fixing (PNF) bacteria can indicate either the biological degradation of agricultural soils or their resistance to agricultural practices (Wolińska et al., 2017b). It has been reported that *Devosia* and *Methylobacterium* representatives are sensitive to agricultural land use, whilst *Burkholderia, Cupriavidus, Mesorhizobium, Microvirga*, and *Phyllobacterium* are resistant to human agricultural practices (Wolińska et al., 2017b). Moreover, our previous studies proved that, besides the mode of land use, soil genesis had an influence on the structure of PNF bacteria and *Bacteroidetes* and their colonization preferences (Wolińska et al., 2017b,c). In general, a higher OTU number was noted in autogenic soils – formed from loess material, rather than in hydrogenic soils – formed under an impact of stagnant water and lithogenic soils – originating from limestone (Wolińska et al. 2017b,c). In the case of *Bacteroidetes*, it was confirmed (Wolińska et al., 2017c) that their sensitivity to agricultural land use was similar, irrespective of the classification system (soil orders and/or soil origin).

Consequently, finding more metagenomic indicators of soil fatigue is possible if bacterial taxonomic classification levels lower than phyla or classes, like families and genera, are taken into account. To our knowledge, this is the first report where biodiversity at the level of families and genera in Polish agricultural soils and wastelands are presented with indication of soil fatigue bacterial indicators. Thus, the objective of the study was to determine new metagenomic indicators demonstrating sensitivity to soil fatigue as an effect of long-term agricultural use, as well as new metagenomic indicators showing bacterial resistance to human agricultural activity.

2. Materials and methods

2.1. Study site and soil sampling

Soil materials were collected in the south-east part of Poland, Lubelskie voivodeship (51° 13′N, 22° 54′E), one of the largest Polish agricultural regions with a surface area of 2515 km^2. The economy of the region is based on agriculture. Lubelskie voivodeship has a borderline humic continental climate with cold and dump winters and warm summers. Importantly, all basic Polish soils types are represented in this region. Thirty-one soil samples were taken for the study. The soils were classified as *Luvisols*, *Arenosols*, *Phaeozem*, *Gleysols*, *Fluvisols* and *Leptosols* (IUSS Working Group WRB, 2006). Additionally, the soil materials were grouped based on the soil formation process into 3 units: (1) autogenic – formed from loess material (including *Luvisols, Arenosols*, and *Phaeozem*) (2) hydrogenic – formed under an effect of stagnant water (including *Gleysols* and *Luvisols*), and (3) lithogenic – formed from limestone (including *Leptosols*).

The soils were sampled according to Polish Norm Rules (PN-R-04031, 1997) dedicated to soil sampling for biological purposes. The agricultural sampling sites were selected on the basis of locations specified in the Bank of Soil Samples (BSS) created in 1991 and belonging to the Institute of Agrophysics, Polish Academy of Sciences in Lublin (Gliński et al., 1991; Bieganowski et al., 2013). Precise localization and description of the agricultural sites catalogued in BSS created a possibility of a precise return to the sampling place (Gliński et al., 1991; Wolińska et al., 2017a). Consequently, the soil cultivation history of each of the studied sites has been documented since 1991, which already allows a conclusion about soil fatigue. From BSS deposits, 16 locations were specified for the Lubelskie voivodeship region (Table 1). The soil samples for metagenomic analysis were collected from agricultural non-rhizospheric soils (at a suitable distance from the plant) in the period before moving the vegetation in order to eliminate the rhizosphere effect on biodiversity. In fact, the quantitative and qualitative compositions of the microbial community were compared in non-rhizospheric trials of agricultural and uncultivated soils. Soil samples taken from uncultivated (non-agricultural soils) were used as controls, but neither pastures nor forest soils were taken into account.

For each of the 16 sample sites, 10 × 10 m squares were selected, and minimum 50 random samples were taken from the surface layer from each site (0–20 cm). Single samples were combined and homogenized into one representative sample. The same pattern was applied while taking control samples located in close proximity to the agricultural soils and belonging to the same soil types as the agricultural soils.

In this manner, 16 samples for agricultural soils (coded A – as agricultural) and 15 for non-cultivated sites (coded C – as control) were obtained. Since they represent the same soil type and originate from the