



## Original Articles

# Defining a reference system for biological indicators of agricultural soil quality in Wallonia, Belgium

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## ABSTRACT

Tools that will enable the assessment of agricultural soil quality and include measurements of biological indicators, such as soil respiration or nitrogen mineralisation, are increasingly in demand. Such tools require the establishment of reference systems to provide comparative ‘baseline’ or ‘normal’ values. In this study, we measured the spatial and seasonal variability of eight biological indicators (including two eco-physiological quotients) in order to establish a reference system at the regional level of Wallonia (Southern Belgium).

Respiration potential, microbial biomass carbon, microbial C/N ratio, net nitrogen mineralisation, metabolic potential of soil bacteria, earthworm abundance, microbial quotient, and metabolic quotient were measured at 60 sites across contrasting agricultural regions (different soil types and climate) in both grasslands and croplands. Additionally, the same biological indicators were measured four times during the vegetation period (April, June, August, and October) in 11 cropland sites to assess seasonal variability. Reference ranges were defined for each biological indicator, based on the addition of variances (seasonal and spatial) and the calculation of cumulative distribution functions.

Land use was the most useful classification variable to define a reference system in Wallonia. Two separate reference systems, one for grasslands and one for croplands, were thus appropriate for Wallonia. Sampling season had a significant effect on all biological indicators. The inclusion of seasonal variability resulted in reference ranges 1.1–5.7 times wider than ranges accounting only for spatial variability. The reference system provides a basis for a first comparative assessment of soil quality for most agricultural soils of Wallonia, independent of sampling period.

## 1. Introduction

Tools for the assessment of soils are needed to evaluate the effects of agricultural practices and support sustainable soil management. Farmers generally rely on a combination of informal observations and chemical analyses to assess the state of their soils (Wood and Litterick, 2017). In Wallonia, a federal entity in the southern part of Belgium, a network of provincial laboratories provides soil analyses for farmers. So far, soil assessment routinely includes a range of different chemical and physical parameters (Genot et al., 2011), but an increasing demand for measures linked to the biological activity of soils has been noted (Genot, personal communication). Furthermore, the possibility of future legal obligations to report on biological parameters of agricultural soils motivates laboratories to prepare for this eventuality and expand their offer.

Biological indicators, such as soil respiration or earthworm abundance, are commonly used in comparative studies, for instance to measure the effects of different soil management practices (D'Hose et al., 2014; Van Leeuwen et al., 2015) or to monitor the recovery of degraded soils (Gilsotres et al., 2005). These indicators have been integrated into soil monitoring networks (SMN) across Europe (van Leeuwen et al., 2017). A pilot study in Wallonia allowed the collection of a first data set on biological indicators (Krüger et al. 2017), but routine measurements of biological indicators are not yet commonly available for farmers. In order to offer such measurements, agricultural laboratories need to select indicators that are economic in terms of running costs, initial investment, and required measurement times (Doran and Zeiss, 2000). Furthermore, the ecological meaning of the data needs to be easily interpretable by managers and farmers. Thus, we considered the use of well-established methods with clear links to soil functions and easily interpretable data, such as microbial

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biomass (Vance et al., 1987) or nitrogen (N) mineralisation, as preferable to novel measurements such as molecular microbial diversity that require scientifically demanding data analysis (Hermans et al., 2017).

While scientific studies include control sites, these are not commonly available for commissioned measurements. Evaluation of results thus requires a comparative reference system, where values are compared to those for other sites across the same farming region. The concept of critical limits (i.e. distinction between safe and unsafe conditions in toxicological contexts; Chaisuksant et al., 1999) cannot be applied to biological indicators. As biological indicators are subject to inherent site factors, such as soil texture, mineralogy (Sparling, 1997) and climate (Wienhold et al., 2009), reference values need to be defined according to a hierarchy of site and landscape classification variables. Following the definition of soil quality as ‘fit for a purpose’, a classification of reference systems according to actual or intended land use is a logical choice (Sparling, 1997). For some biological indicators, areas might be further divided based on their driving factors (texture, pH, soil organic carbon content) (Dequiedt et al., 2011; Griffiths et al., 2011). In the context of SMNs, criteria to divide the data into subsets vary between European countries: for instance, the online database of ranges of biological indicators measured on 47 sites in the French “Bioindicator program” allows division of sites by land use, contamination level, texture, pH, and soil organic carbon content (Cathelineau et al. 2014, Pérès et al., 2011), whereas the French SMN RMQS bases its approach on land use, management system, fertilisation intensity, tillage, use of pesticides (Cluzeau et al., 2012), and the Dutch SMN BISQ (Biological Indicator system for Soil Quality) uses a stringent combination of soil type and land use to stratify its sampling sites (Rutgers et al., 2009). Given the similarity in pedoclimatic conditions between Wallonia and its neighbouring countries, a similar approach to stratification could prove useful.

Spatio-temporal variability presents a challenge for defining value ranges in reference systems. Biological indicators reflect complex interactions between different environmental parameters (Ritz et al., 2009), and, generally show a higher spatiotemporal variability than physical and chemical indicators. Spatial variability can be attenuated to some degree through the use of composite samples, sometimes made up of several hundred cores (Bloem et al., 2005). In Wallonia, soil assessment for farmers is generally provided for composite samples, representative for homogenous farm plots (similar in colour, texture, rock content, humidity, etc.), following ISO norms (Genot et al., 2012).

While shorter reaction times to environmental changes is one of the motivations for the inclusion of biological indicators in SMNs (Dale and Beyeler, 2001), seasonal variation of soil conditions (substrate availability, temperature, moisture etc.) can hamper their interpretation (Schloter et al., 2003). In SMNs, the sampling moment is generally set, taking into account management practices and meteorological conditions in order to reduce inter-annual variation. Samplings should be performed before ploughing, in the absence of recent frost or fertilization, in humid, but not waterlogged soil. As such, both sampling in spring or autumn are considered as suitable (Bloem et al., 2005). In practice, the sampling period often depends on the farmers’ wishes that soils are sampled before seedlings might be disturbed. Nonetheless, the variability of weather conditions in the Atlantic climate (Zveryaev, 2004) might result in highly different conditions between years and subsequent differences in biological indicator measurements, even if criteria for suitable sampling conditions are clearly defined. Soil fauna indicators are generally very sensitive to meteorological conditions, whereas soil samples for molecular analysis are sampled year-round in France. Samples for microbial analysis can be pre-incubated to mitigate the effect of weather conditions preceding sampling (Bloem et al., 2005), but this might also impact results due to substrate depletion in pre-incubated samples. Additionally, changing crop rotations and related management practices result in soils where the underlying dynamics are difficult to disentangle, if the land management history can be documented at all. While it is possible to define reference plots without taking into account the variability in that location for comparison (Rutgers et al., 2009), we considered the added value of including spatiotemporal variability in the reference system important for its practical use as a meaningful assessment tool.

The main goal of a SMN is to track the long-term evolution of soil quality at specific sites over years or even decades (Mol et al., 1998). Measurements from representative sites can also provide reference values against which data from other sites can be compared. Representativeness of data sets collected within a SMN is defined, first through the selection of sampling sites (generally chosen through a regular grid or a stratification approach (Morvan et al., 2008)), the standardisation of sampling and measurement procedures (including sampling period), and through the mathematical approach used to express the ranges. To facilitate diagnosis, ranges are expressed as quartiles (as done in France, (Cluzeau et al., 2012)), or through calculation of 95% confidence ellipsoids referred to as the Normal Operating Range (NOR) (used in the Netherlands, (Kersting, 1984; Pereira e Silva et al., 2013)). NOR also present the advantage of mathematically combining several measurements. These approaches account for spatiotemporal variability of biological indicators, without giving extreme values power to skew the data distribution. Such methods require large data volumes that are so far not available in Wallonia.

The development of a reference system for biological indicators of soil quality for Wallonia was the main aim of this study. Specifically, the objectives of this study were to (1) assess the relevant classification variables for defining reference values (landscape classification and soil chemical parameters); (2) quantify the relative importance of seasonal and spatial variability; (3) define a representative reference system, accounting for spatial and seasonal variability.

## 2. Material and methods

### 2.1. Site selection and soil sampling

Spatial variability of biological soil quality indicators was studied at the regional scale of Wallonia, south Belgium. Sixty sites from the CARBOSOL network, monitoring soil organic carbon stocks and dynamics in Wallonia (Goedts and van Wesemael, 2007), were selected among 10 landscape units (LSU) through the Latin hypercube method (Minasy and McBratney, 2006) and sampled in spring 2015 (Fig. 1). Soils within each LSU are homogenous with regard to land-use (grassland or cropland), soil type (texture, rock fragment content, and drainage), and belong to the same agricultural region (a proxy for climatic conditions, Table 1). The ten selected LSU represent an area that covers about 47% of the agricultural land of Wallonia and present different conditions along environmental gradients (Chartin et al., 2017). Sites are situated at altitudes of about 60 to 440 m asl. Agricultural practice and current crop are not defined in the thus created CARBIOSOL network for biological indicators, resulting in diverse management situations at the time of sampling.

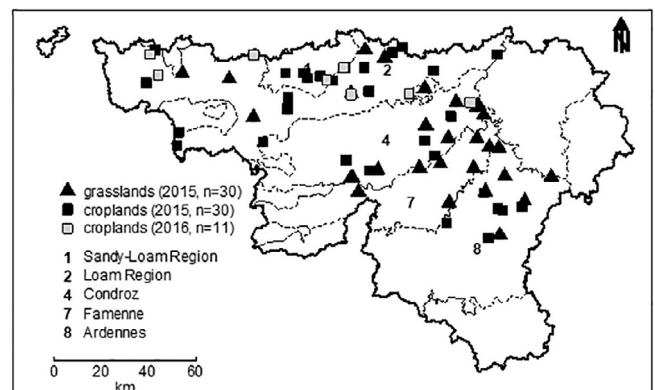


Fig. 1. Location of sampling sites in Southern Belgium (Wallonia). Samples taken to study spatial variability in 2015 are shown in black and samples taken to study seasonal variability in 2016 are shown in grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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