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Enzymatic functional stability of Zn-contaminated field-collected soils: An ecotoxicological perspective

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HIGHLIGHTS

- An optimized enzymatic functional stability (FS) approach was developed.
- Twenty metal-contaminated field-collected soils and four enzymes were tested.
- This original approach includes the site history within the FS scores.
- This new FS approach was compared to other FS scores available in the literature.
- This new FS approach better assessed the Zn toxicity of the soils.

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ABSTRACT

Functional stability (FS) is an ecosystem attribute that is increasingly promoted in soil health assessment. However, FS is currently assessed comparatively, and it is therefore impossible to generate toxicity parameters. Additionally, the FS scores in the literature do not consider site and contamination history within the score. To address these issues, three new FS scores adapted to an ecotoxicological context and based on the Relative Soil Stability Index (RSSI) method were developed. The aim of the study was then to determine the FS score(s) that best describe the toxicity of metal-contaminated field-collected soils. Twenty pairs of Zn-contaminated soils (contaminated and reference soils) were collected on the field, and their enzymatic FS (arylsulfatase, protease, phosphatase and urease) and metal fractions (total and bioavailable) were analyzed. New RSSI-based and existing FS scores were calculated for each enzyme and correlated to the Zn fractions. One of the new RSSI-based scores was well correlated with the bioavailable labile Zn concentration for the arylsulfatase, phosphatase and urease (coefficients of regression higher than 0.50). Furthermore, this FS score was not affected by the soil organic matter and depended little on other soil properties. Other FS scores were correlated to labile Zn for only one enzyme, which varied according to the score. The new RSSI-based score thus better attributed Zn toxicity to field-collected soils than other FS scores.

1. Introduction

Microbial ecology research is at a tipping point at which ecosystem health and ecotoxicology are increasingly assessed by attributes, ecosystem services or ecologically relevant features rather than conventional biological indicators (Garbisu et al., 2011). Understanding how ecosystems respond when faced with additional stressors is an example of these attributes known as functional stability (FS) or engineering resilience (Griffiths and Philippot, 2013). In recent years, many authors have endorsed FS assessment as a holistic, timely and relevant approach to protect ecosystems facing simultaneous stressors (Pereira e Silva et al., 2013; Shade et al., 2012; Zell and Hubbart, 2013). Griffiths and Philippot (2013) recently described and supported this approach in a complete review, showing that over 40 soil studies based on FS assessment were published in the last decade. The soils were mostly pre-stressed by natural or anthropogenic stressors such as wild fire episodes, land management or contamination. Studies on pre-stressed ecosystems constitute a particular field known as co-tolerance (Tobor-Kapłon et al., 2006b; Vinebrooke et al., 2004). One third of the studies recorded in the review of Griffiths and Philippot (2013) assessed the co-tolerance of pre-stressed metal contaminated soils through FS assessment, suggesting that an FS approach is praised and well-suited for studying multi-stressed and tolerant ecosystems.

The way for expressing the FS varies widely in the literature. For example, certain studies plotted and visually compared microbial response after the laboratory-controlled (LC) stressor (Deng et al., 2009; Philippot et al., 2008). However, a comparison of numerous graphics

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is neither convenient nor objective, since each soil has its own site history conferring various biological activity baselines that depend on physicochemical properties and background metal concentrations. To simplify and organize the data treatment, the FS information is often gathered in static spot-time scores right after (resistance score) and/or at a specific time following the LC stressor (resilience score) (Chaer et al., 2009; Epelde et al., 2012; Griffiths et al., 2000; Mertens et al., 2010; Orwin and Wardle, 2004; Tobor-Kapłon et al., 2006a). Bécaert et al. (2006) innovated by considering the dynamic of the biological function after a heat stressor and developed the Relative Soil Stability Index (RSSI) method, the first time-integrated method. Zhang et al. (2010) followed this approach to propose a time-integrated resilience score similar to the RSSI score. Table 1 shows the equations for the FS scores that are currently available.

The RSSI method is particularly interesting in ecotoxicity assessment since it shows 2,4-D and copper toxicity in soil enzymes by a decrease in RSSI scores when soil is contaminated, considering that a high RSSI score implies high FS (Bécaert et al., 2006; Dussault et al., 2008). Furthermore, the RSSI method monitors the activity of various soil enzymes, thus providing many advantages: it gives insight into the main biogeochemical cycles, and soil enzymes are mainly produced by indigenous microbial community and are metal-sensitive, easily measurable, affordable and reproducible (Caldwell, 2005). Additionally, FS studies related to the biological activity of a microbial community may be more reliable than those related to microbial abundance or structural diversity. Indeed, these two last categories of microbial indicators may be modified by a stressor without affecting the overall functional integrity of the soil microbial community due to its high level of functional redundancy (Epelde et al., 2012). Moreover, the RSSI method uses 60 °C heating as an LC stressor—a temperature slightly above the optimal temperature range of mesophile microbes and soil enzymes—thus disturbing the enzymatic activity of the mesophile microbial community but allowing enzymatic resilience in most cases (Bécaert et al., 2006). These characteristics all make the RSSI method better suited to assessing the FS of metal-contaminated field soils.

However, past RSSI studies used spiked soils instead of field-contaminated soils and few soils were tested (Bécaert et al., 2006; Dussault et al., 2008). Furthermore, RSSI and any other FS methods are comparative: the score of a degraded soil is compared to the score of a reference/control soil. This approach by category is not suitable to assess metal toxicity since any causal relationship can be obtained from a comparative approach, meaning that any toxicity threshold could be calculated. An alternative could be to correlate FS scores to the contaminant concentration in order to obtain a causal relationship, which, in turn, generates regression parameters such as toxicity thresholds. However, no one to our knowledge has tested this approach. Still, this causal relationship implies that FS variability must chiefly be explained by the contaminant concentration and not by other soil properties—a concept seldom explored until now. Most of the effects of soil properties on FS were studied in agricultural ecosystems, and few were studied in contaminated soils (Chaer et al., 2009; Dussault et al., 2008; Gregory et al., 2009; Kuan et al., 2007; Zhang et al., 2013). In any case, one important issue remains: RSSI and all other available FS scores do not take into account the site or contamination history within the score, i.e. the background physicochemical and biological influence of the sampled site, since the contaminated disturbed site is not normalized by the uncontaminated/reference soil. In other words, there is one FS score for the contaminated soil (time-integrated contaminated disturbed soil divided by time-integrated contaminated undisturbed soil) and one FS score for the reference soil (time-integrated reference disturbed soil divided by time-integrated reference undisturbed soil, see Table 1). Using this approach, different terrestrial sites could not be compared since background enzymatic activity is dependent on site physicochemistry (Vangheluwe et al., 2007).

To fill this gap, a new RSSI-based approach including three new RSSI-based scores adapted to a contamination context is proposed in this study. The aim is to determine which FS scores better assess the toxicity of metal-contaminated field soils. First, FS scores (existing and new ones) will be correlated to metal fractions in order to obtain causal relationships between FS and metal contamination and identify the best-suited FS score(s) for a metal contamination context. Then, the effect of soil physicochemical properties on the FS score will be analyzed in order to verify whether FS variability is better explained by soil properties other than metal concentration. This study was a preliminary work of a subsequent study which determined whether an enzyme-based functional diversity index is a suitable ecotoxicological indicator of long-term Zn contamination in field-collected soils (Lessard et al., 2014). Zinc was chosen for this study because several monometallic contaminated field-collected soils of various Zn concentrations were available and also because Zn is a co-factor of several soil hydrolytic

Table 1
Description of functional stability scores used in this study.

Properties			FS score ID		Description	FS equation related to reference soil[a]	FS equation related to contaminated soil[a]
Existing scores	Two scores per pair of soils comparing reference soil to contaminated soil	Dynamic (integral)	#1	Bécaert (Bécaert et al., 2006)	Original RSSI score integrated over 11 days	$\frac{\int_1^{11} R_D(t)dt}{\int_1^{11} R_{UD}(t)dt}$	$\frac{\int_1^{11} C_D(t)dt}{\int_1^{11} C_{UD}(t)dt}$
			#2	Zhang (Zhang et al., 2010)	Zhang resilience score integrated over 11 days	$\int_1^{11} \frac{\left(\frac{R_D(t)}{R_{UD}(t)}\right) \times 100}{(11-1)} dt$	$\int_1^{11} \frac{\left(\frac{C_D(t)}{C_{UD}(t)}\right) \times 100}{(11-1)} dt$
		Static (spot time)	#3	Griffiths (Griffiths et al., 2000)	Griffiths resilience score at day 11	$\left(R_{UD}(11) - {}^{R_{UD}(11)}/_{R_D(11)}\right) \times 100$	$\left(C_{UD}(11) - {}^{C_{UD}(11)}/_{C_D(11)}\right) \times 100$
			#4	Chaer (Chaer et al., 2009)	Chaer score at day 11	$\left\lVert {}^{R_D(11)}/_{R_{UD}(11)} - 1 \right\rVert \times 100$	$\left\lVert {}^{C_D(11)}/_{C_{UD}(11)} - 1 \right\rVert \times 100$
			#5	Orwin & Wardle (Orwin and Wardle, 2004)	Orwin & Wardle resilience score at day 11	$\left(\frac{2(\lvert R_{UD}(0) - R_D(1)\rvert)}{\lvert R_{UD}(0) - R_D(1)\rvert + \lvert R_{UD}(11) - R_D(11)\rvert}\right) - 1$	$\left(\frac{2(\lvert C_{UD}(0) - C_D(1)\rvert)}{\lvert C_{UD}(0) - C(1)\rvert + \lvert C_{UD}(11) - C_D(11)\rvert}\right) - 1$
New score proposed	One score per pair of soils including the site and contamination history	Dynamic (integral)	#6	$RSSI - a$	New RSSI-based score integrated over 11 days	$\frac{\int_1^{11} C_D(t)dt}{\int_1^{11} R_D(t)dt}$	
			#7	$RSSI - b$	New RSSI-based score integrated over 11 days	$\frac{\int_1^{11} C_D(t)dt}{\int_1^{11} R_{UD}(t)dt}$	
			#8	$RSSI - c$	New RSSI-based score integrated over 11 days	$\frac{\int_1^{11} \left(\frac{C_D(t)}{C_{UD}(t)}\right) dt}{\int_1^{11} \left(\frac{R_D(t)}{R_{UD}(t)}\right) dt}$	

[a] C = contaminated soil, R = reference soil, D = disturbed, UD = undisturbed, (t) = integrated over time, Sample (x) = sample measured at day x.