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MINIREVIEW

Standardisation of methods in soil microbiology: progress and challenges

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Abstract

A plethora of methods have been developed over the few last decades to enable a better understanding of the ecology of soil microbial communities and their roles in soil functioning. However, there is generally considerable variation (both subtle and more extensive) in the actual realisation of these methods, and limited efforts have been devoted to their standardisation, despite this being crucial to underpin data comparison and integration. Ensuring comparable data across studies through standardisation is arguably best-practice, as well as necessary to effectively meet the objectives of various schemas, which require assessment of the consequences of the global change and intensification of human activities on the functioning of the soil ecosystem and its biological diversity. This article presents an overview of the existing and forthcoming ISO standards in soil microbiology and highlights possible future research efforts to be undertaken for developing new standards. We also discuss some practical and theoretical bottlenecks and hurdles that have limited standardisation in soil microbiology up to now.

Introduction

Microorganisms in soil ecosystems are ubiquitous, abundant, diverse and essential for many soil functions such as carbon and nitrogen cycling, plant productivity and climate regulation (Whitman *et al.*, 1998; Torsvik *et al.*, 2002; Falkowski *et al.*, 2008; van der Heijden *et al.*, 2008; Bodelier, 2011). Because of their importance, there is a large volume of past and contemporary researches that aims to understand the ecology of soil microbial communities, with thousands of articles devoted to this research field published annually. Numerous methods have been developed to estimate abundance, diversity and activity of soil microorganisms. Several such procedures are now successfully applied on a regular and on-going basis, perhaps most notably the chloroform fumigation-extraction technique for estimating microbial biomass (Vance *et al.*, 1987), and DNA fingerprinting approaches for estimating the structure of microbial communities. Perversely, many of these methods become victims of their own success, and a plethora of laboratory- or even user-specific protocols, which contain minor to major modifications of the initially described methods, are now used worldwide. However, these differences between protocols are far from being inconsequential as they often include inherent bias, which hamper data comparison across studies, let alone laboratories. Indeed, variations in data obtained by different laboratories or using different protocols are commonly reported (Ocio & Brookes, 1990; Beck *et al.*, 1997; Krsek & Wellington, 1999; Martin-Laurent *et al.*, 2001; Creamer *et al.*, 2009; Pan *et al.*, 2010). A theoretically obvious, albeit practically challenging, solution is to define and use standardised methods. This is becoming all the more important because an exponentially increasing volume of data is now being generated, particularly with the advent of automated or high-throughput techniques, notably in relation to molecular biology. Such techniques offer exciting opportunities for better understanding soil microbial diversity, how it relates to soil functions, and more effective ways to manage terrestrial ecosystems to meet the challenges of sustainability. This grand challenge should be facilitated by ensuring compa-

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rable data, which is necessary in order that our knowledge of soil microbial communities can be effectively integrated.

The concept, and practice, of standardisation in soil microbiological assays can be applied at a range of levels, from the individual researcher/group (vital to ensure coherence within a body of experimentation), through institutional (assists integration and coherence within institutional-level programmes), to national (e.g. British Standards and French National Organisation for Standardisation) and international [e.g. International Organisation for Standardisation, (ISO)]. Here we focus on the latter context, as this is arguably the most effective route to achieve the higher-level aims of standardisation. Moreover, science itself is an international collaborative effort and comparisons across studies need to be performed beyond country borders, not least because soils and the organisms they support operate entirely independently of such boundaries. Standards providing internationally agreed methods for assessing soil microorganisms have mostly been developed by the International Organisation for Standardisation (ISO). However, the number of ISO standardised methods is still scant in relation to the numerous methods that have been developed within the field of soil microbiology. In addition, the use of ISO methods in soil microbiology research articles, outside of ecotoxicology studies, is in our perception relatively rare. In this article, we underline the importance of standardisation in soil microbiology, present an overview of the existing and forthcoming ISO standards, and discuss some technical and cultural hurdles. One aim is to stimulate debate in this field and to encourage a move toward the development and greater dissemination of internationally agreed standards in soil microbiology.

Standardisation in soil microbiology: dealing with the natural complexity and diversity

Soils are arguably the most complex systems on the planet, given the extraordinary diversity of their chemical and biological constituents, as well as the extreme structural heterogeneity (Ritz, 2008). There are also a wide range of soil types, with huge numbers of classes of soil recognised in taxonomic schemes both at global down to national scales, for example, some 748 Soil Series are recognised in the Soil Survey of England and Wales (Clayden & Hollis, 1984) and thousands of types in the lower-order taxa of World Reference Base (FAO, 2006). The geo-spatial distribution of soils is also complex across virtually all size scales, which means that studies at almost any spatial scale involve a variety of soil types, which may confound the ready application of standard techniques. This diversity of constitution and basic characteristics severely challenges the ability to set standards in measuring soil properties and processes. This is particularly true for biological aspects of soil systems, and in part accounts for the concomitant diversity in methodological variants. Even something as outwardly straightforward as determining soil organic carbon is confounded by the fact that soils can vary from essentially 0–100% organic matter, there is potential (and variable) interference from inorganic forms of carbon, and the same procedure is certainly not appropriate for soils at the two extremes (Nelson & Sommers, 1996). It is often then the case that no single method is universally appropriate and that variants within methods are needed to compensate for differences in properties that may occur if they are to be applicable to the gamut of soils. For example, measuring soil respiration by CO_2 emission is relatively straightforward if the pH of the soil is lower than 7.5, but in more alkaline soils, the partition coefficient of CO_2 between air and water starts to confound the technique because proportionately more CO_2 will prevail in the pore water (Anderson, 1982). The quality and quantity of organic matter and clay vary between soils that affects the nature and extent of potential absorption of biochemicals, notably nucleic acids, such that a range of devices to counter such effects need to be applied, contingent on the soil. These factors can be compensated for by variants in technique, and such variants can be duly standardised. In principle, such matters do not then preclude the *setting* of standards, but they certainly prevent the setting of *simple* standards. Furthermore, there is a significant issue that affects data comparability, as with complex protocols, there is an increased likelihood that different operators will determine different absolute values for measurements, because of accumulations of even subtle differences between each of the steps in such procedures.

Another factor arising from the need for sophisticated/adjusted/complex protocols is the ease with such protocols are agreed upon within the context of a standards setting framework, particularly an international one. This is because the optimal procedures are not necessarily readily defined and can become more a matter of best judgement. For example, it can be argued either way that the pH of the buffer medium in enzyme assays should be standardised to a particular pH, or the pH of the particular soil under scrutiny (German *et al.*, 2011), but there are then supplementary issues of how to determine that pH. Another concern is at which temperature one should measure soil respiration? The same for a sub-arctic tundra soil as one from Namibia or a 'locally pertinent' temperature? And then what moisture content is optimal for respiration measurements and how should that be determined? Such questions are undoubtedly very important

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in defining standards but challenge the attainment of scientific consensus.

Current standards in soil microbiology

Despite the inherent complexity and diversity of soils described earlier, some methods to study soil microorganisms have been standardised since 1997 (Table 1). Due to a strong concern regarding the degradation of soils in relation to local and diffuse contamination or loss of biodiversity, the existing standards were developed by the 'Soil quality' Technical Committee ISO/TC 190 with a strong focus on assessing the effects of chemicals and pollution on the soil fauna and soil microorganisms (Nortcliff, 2002). Methods for measuring soil microbial biomass using substrate-induced respiration and fumigation-extraction were the first ones to be standardised in the field of soil microbiology in the late nineties (ISO 14240, Table 1). Indeed, these methods based on pioneering work of Vance *et al.* (1987) were proposed to provide a sensitive indicator for measuring changes in the total quantity of soil microorganisms in response to environ-

Table 1. ISO standardised methods in soil microbiology

Year	Method	ISO reference	Bibliography
1997	Determination of soil microbial biomass – part 1: substrate-induced respiration method	ISO 14240-1	Jenkinson & Powlson (1976); Anderson & Domsch (1978)
1997	Determination of soil microbial biomass – part 2: fumigation-extraction method	ISO 14240-2	Brookes *et al.* (1985); Vance *et al.* (1987); Ocio & Brookes (1990); Sparling *et al.* (1990); Wu *et al.* (1990); Inubushi *et al.* (1991); Mueller *et al.* (1992); Harden *et al.* (1993a, b)
1997	Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes	ISO 14238	Bremner (1965); Henriksen & Selmer-Olsen (1970); Selmer-Olsen (1971); Stanford & Smith (1972); Andersch & Anderson (1991)
2002	Determination of abundance and activity of soil microflora using respiration curves	ISO 17155	Anderson & Domsch (1978); Nordgren *et al.* (1988); Arnebrant & Schnurer (1990); Chander & Brookes (1991); VanBeelen *et al.* (1991); Stenstrom *et al.* (1998); Wilke *et al.* (1998)
2002	Soil quality – guidance on laboratory testing for biodegradation of organic chemicals in soil under anaerobic conditions	ISO 15473	Beland *et al.* (1974); Gowda & Sethunathan (1976); Healy & Young (1979); Attaway *et al.* (1982); Kearney (1982); Shelton & Tiedje (1984); Ward (1986); Alef & Nannipieri (1995)
2002	Laboratory methods for determination of microbial soil respiration	ISO 16072	Gupta & Singh (1977); Nordgren (1988); Watts *et al.* (2000)
2004[UR]	Determination of potential nitrification and inhibition of nitrification – rapid test by ammonium oxidation	ISO 15685	Belser & Mays (1980); Hansson *et al.* (1991); Stenberg *et al.* (1998); Winkel *et al.* (1999)
2005	Determination of dehydrogenase activity in soils – part 1: method using triphenyltetrazolium chloride (TTC)	ISO 23753-1	Thalmann (1968); Glathe & Thalmann (1970); Wilke (1982); Ohlinger (1995)
2005	Determination of dehydrogenase activity in soils – part 2: method using iodotetrazolium chloride (INT)	ISO 23753-2	Thalmann (1968); Glathe & Thalmann (1970); vonMersi & Schinner (1991); Spothelfer-Magaña *et al.* (1993); Fuchs *et al.* (1994); Ohlinger (1995)
2010	Measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates	ISO 22939	Tabatabai (1994); Stemmer *et al.* (1998); Marx *et al.* (2001); Vepsäläinen *et al.* (2001, 2004); Marx *et al.* (2005); Niemi & Vepsalainen (2005)
2010	Determination of soil microbial diversity – part 1: method by PLFA analysis and PLEL analysis	ISO 29843-1	Blight & Dyer (1959); White *et al.* (1979); Findlay *et al.* (1990); Frostegård *et al.* (1991); Zelles & Bai (1993); Alef & Nannipieri (1995); Zelles (1999); Gattinger *et al.* (2003)
2011	Determination of soil microbial diversity – part 2: method by PLFA analysis using the 'simple PLFA extraction method'	ISO 29843-2	Blight & Dyer (1959); White *et al.* (1979); Zelles & Bai (1993); Gattinger *et al.* (2003)
2011[UP]	Method to directly extract DNA from soil samples	ISO 11063	Tsai & Olson (1991); Smalla *et al.* (1993); Zhou *et al.* (1996); van Elsas *et al.* (2000); Martin-Laurent *et al.* (2001); Niemi *et al.* (2001)

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mental factors or anthropogenic disturbances. Most of the other existing ISO standards were developed for similar purposes and are therefore biased toward effective monitoring of the soil microbial community to meet extant policy requirements (Table 1). This trend is particularly obvious for ISO 14238 'Determination of nitrogen mineralisation and nitrification in soils and the influence of chemical on these processes' and ISO 15473 'Testing for biodegradation of organic chemicals in soil'. Thus, ISO 14238 was designed to determine the effects of different concentrations of a chemical on the N-cycling processes using dose–response curves while ISO 15473 gives general guidelines for the selection and method of tests to determine the biological degradation of organic chemicals introduced into the soil either intentionally or accidentally.

Criteria related to applicability and effectiveness of standards for routine analyses such as high throughput analysis, cost, usability or data interpretation have up to now excluded molecular methods, such as terminal fragment length polymorphism for assessing microbial diversity, despite their widespread use in research. However, among the new ISO standards, the development of the ISO 11063 standard for soil DNA extraction (Petric *et al.*, 2011) is of special interest because it is the first step of all PCR-, hybridisation, and sequencing-based molecular analyses of the diversity and abundance of soil microbial communities. As a result, thousands of studies are performed yearly in environmental microbiology using soil DNA extraction methods. Due to this important business market, at least ten companies are commercialising soil DNA extraction kits, which add to the list of home-made protocol. This is despite it being well established that the apparent microbial diversity determined by any nucleic acid analysis procedure is contingent on the DNA extraction method (Frostegård *et al.*, 1999; Martin-Laurent *et al.*, 2001; deLiphtay *et al.*, 2004; Feinstein *et al.*, 2009; Pan *et al.*, 2010; Delmont *et al.*, 2011). The ISO 11063 standard for soil DNA extraction is based on both chemical and physical approaches for extraction and lyses of the microbial cells as described by Petric *et al.* (2011). This ISO is timely since studies of soil microbial diversity based on soil DNA extraction are generating an exponential amount of sequence data, and large scale projects aiming at sequencing the soil metagenome are now launched (Vogel *et al.*, 2009). Knowledge of the identity and the quantity of each compound used in the ISO 11063 or any ISO protocol provides transparency and allow users a complete quality control, which is a major advantage over commercial kits. Thus, production batch effects can occur, and this has been observed for some commercial soil DNA extraction kits (unpublished data). A transparent protocol also avoids the risk of subsequent modifications of the kit reagents by companies or risks associated to the versatility of their business strategies such acquisition and merging, which are common activities for biotechnology industry.

While no nucleic acid-based method for assessing soil microbial diversity have yet been proposed for international standardisation, two lipid-based methods have recently became ISO standards (Table 1). Phospholipid fatty acid (PLFA) and phospholipid ether lipids (PLEL) analyses are rapid and inexpensive methods for providing a quantitative measure of the viable soil biomass and complex microbial community profiles. They offer the advantage of targeting the entire microbial community, thus allowing calculation of the fungal/bacteria ratio using markers PLFA specific of these domains (Frostegård & Bååth, 1996). Since the late 1990s, several comprehensive reviews discussing the strengths and weaknesses of the use of lipid fatty acids for assessing microbial biomass and community structure in soil have been published (Olsson, 1999; Zelles, 1999; Kaur *et al.*, 2005; Frostegård *et al.*, 2011). Unfortunately, while some of the ISO standards described in Table 1 have been published more than 10 years ago, their use by the scientific community is still very limited. Thus, the ISO has no power to enforce the implementation of the standards it develops and therefore adoption of the ISO standard is still mainly voluntary.

Directions for future standards

The standardisation effort is uneven between methods addressing the abundance, the diversity and the activity of the soil microbial community. Indeed, while there are already three ISO standards for quantifying soil microbial biomass, a new work item proposing a standard to estimate the abundance of the soil bacterial community by 16S rRNA gene targeted quantitative PCR (qPCR) was recently adopted by the Soil quality ISO technical committee (Australia, September 2011). The recent developments of qPCR analyses also allow the quantification of the abundances of specific functional or taxonomical microbial groups, which may represent useful bioindicators (Wessen & Hallin, 2011). With the use of appropriate blanks, internal and surrogate standards, qPCR is a reliable method having the advantage to offer high throughput and cost-effective analyses.

For a better understanding of soil microbial activity, or more generally of soil functioning, several methods for quantifying potential enzyme activity have been developed. Even though these methods providing an insight of the size of the enzyme pool have some limits (Wallenstein & Weintraub, 2008), they are commonly used as microbiological indicators of soil quality and should therefore be

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standardised for comparison of microbial activities both between soils and laboratories. For example, because of their environmental and agronomical importance, microorganisms involved in N-cycling are of key interest. In addition, they are popular models in soil microbial ecology for relating microbial diversity and soil functioning. However, only measurement of potential nitrification has been internationally standardised up to now, while methods for monitoring other N-processes such as nitrogen fixation and denitrification also necessitate standardisation. For example, the original protocol for estimating potential denitrification (Smith & Tiedje, 1979) has been modified in many ways. In this assay, to measure the activity of the pool of denitrification enzymes in the soil at the time of sampling, soil slurries are incubated in the laboratory in non-limiting denitrification conditions (without oxygen, addition of nitrate and carbon, and of chloramphenicol to avoid *de novo* synthesis) so that only the amount of enzyme is rate-limiting. Changes in the original protocol include excluding the chloramphenicol, which can decrease the activity of synthesized enzymes, addition of different carbon types and amount (glucose, acetate, glumatic acid, etc) and incubation of the soil slurries in various conditions. Similarly, determination of the nitrogenase activity using the acetylene reduction technique (Hardy *et al.*, 1968) is subjected to various modifications of the protocol resulting, for example, in variants of the acetylene concentration (0.03–0.1 v/v). In contrast to other methods, most modifications of these methods are not soil-specific and both potential denitrification and nitrogen-fixation assays could readily be standardised in future.

Finally, regarding methods to monitor the diversity and the structure of the soil microbial community, the adoption of the ISO 29843 for PLFA and PLEL analyses opens the path for other standards. While it is too early to propose any standardisation of the new high-throughput sequencing technologies (e.g. 454 pyrosequencing, etc…), other powerful approaches such as those based on taxonomic and functional microarrays meet the criteria to become standards. Of course these perspectives for the development of future standards in soil microbiology are not exhaustive, and we encourage soil microbiologists to expand it by proposing other popular methods for standardisation.

The ISO standardisation process

If one is interested in developing new international standards, it is worth reviewing how standards are developed within the ISO framework. According to ISO, a standard is a document that is established by consensus and approved by a recognised body (ISO/IEC, 2004). It provides, for common and repeated use, rules, guidelines or characteristics for activities or their results, aimed at the achievement of the optimum degree of order in a given context. Standards should be based on the consolidated results of science, technology and experience, and aimed at the promotion of optimum community benefits (ISO/IEC, 2004). Different types of standards can be developed within this framework (e.g. terminology, product, process, service, testing standards). Such standards are elaborated by technical committees and/or subcommittees that usually comprise representatives from the industrial, technical, business sectors as well as representatives of government agencies, testing laboratories, consumer associations, non-governmental organizations and academia.

The standardisation process includes six successive stages, taking place over a time period usually not exceeding 48 months: *viz.* proposal, preparatory, committee, enquiry, approval and publication stages (ISO/IEC, 2009) (Fig. 1). To confirm the need for the development of a new standard, the new work item proposal should be supported by scientific papers presenting the scientific background, and some results demonstrating the applicability and the relevance of the method. A proposal is accepted when at least five participating countries vote positively and nominate experts to participate actively in

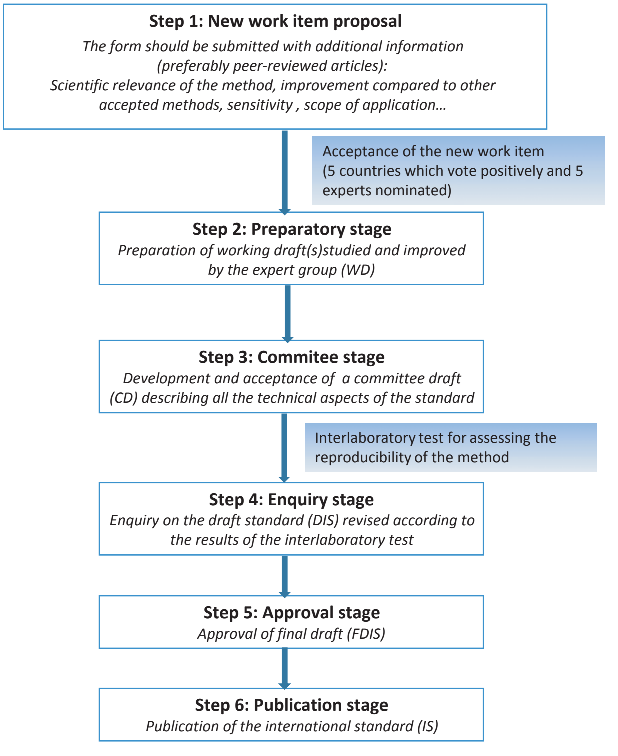

Fig. 1. Flow chart summarising the different steps for standardising a new test method within the ISO framework.

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its development. The first draft of the method is submitted to the experts for discussion and improvements until a consensus has been reached on the technical content. Then, the draft document is distributed for voting and comments by the participating countries of the technical or sub-committees. In case of major disagreements, successive committee drafts may be considered before submission of the text as a draft international standard.

The validation process of a future standard is crucial before publication as an international standard. It involves laboratories from National Bodies of the relevant technical or sub-committees (but not exclusively) for evaluation of the reproducibility of the test method under standardisation. The resulting performance characteristics of this inter-laboratory trial are part of the standard. When all due processes have been satisfactorily completed, the standard is then officially published and released for adoption. International standards are then reviewed at the least 3 years after publication and every 5 years after the first review by all the ISO member bodies to incorporate, in particular, improvements of the method or technical changes. During this review process, members of the technical or sub-committees decide whether the standard should be confirmed, revised or withdrawn.

Fictitious, cultural and real hurdles

As underlined by Pan *et al.* (2010), inter-calibration of protocols is not a common practice in environmental microbiology. As a consequence, while an impressive list of methods, regularly summarised in books, has been developed for studying microorganisms in soils, limited effort has been devoted to standardisation. This paradox is accentuated by the fact that most of these methods are subjected to almost endless modifications of their protocols, which can affect the results and hamper data comparison. These subtle to deep changes can be as a result of weaknesses in the original protocols, which are often related to a failure when applied to a different soil. However, a large number of variations in protocols can still be found in the literature for similar or even identical soils. One could therefore ask whether the existence of so many deviating protocols only reflects a true need for modifications because of the overwhelming diversity and complexity of the soils, or if there are other factors involved conveyed by a certain lack of rigor.

Possibly the fact that soil microbiology is still facing an tremendous and ongoing method development can be considered as contradictory to developing standards. However, evolving fields with technological evolution, new methods or new quality and safety requirements are not an obstacle to standardisation. Indeed, in biomedical science, laboratory-based medical and scientific microbiologists from throughout the Health Protection Agency in Scotland have developed the National Standards Methods, which include, for example, a standard for the detection of influenza viruses by qPCR. Within the ISO, all existing standards are reviewed at intervals of not more than 5 years to evaluate whether a revision is required. This is, for example, the case of the ISO 15685 ‘Determination of potential nitrification and inhibition of nitrification – rapid test by ammonium oxidation’, which was revised in 2011.

Another obstacle could be the naïve thinking that certain of our methods are inadequate for standardisation. It is essential that standardised methods provide meaningful information, but not that they are ‘perfect’. In soil microbiology, such perfection would apply to an assay that provides a true picture of microorganisms’ activity, diversity or abundance in the soil. Given the complexity of the soil system and inherent biodiversity, this may in any case be untenable. As the accuracy of any method in soil microbiology cannot be estimated directly but only through the prism of other methods, microbiologists are facing a potentially unsolvable paradox. In addition, sample-specific optimisation of methods can lead to ‘near-sightedness’, the more detailed description of the studied soil being at the price of not seeing the bigger picture because of the impossibility to compare and integrate data across studies.

Evaluation of the best protocol to standardise is also often hampered by a trade-off situation in which one advantage is lost for another. An example of such a circumstance is the trade-off in relation to soil DNA extraction where the DNA yield can be increased, but typically at the cost of lower quality which may then compromise its apparent representativity, particularly where annealing processes are important.

Final remarks

In the recent years, increasing efforts have been made to promote consistency among laboratories. These efforts were mostly devoted to improving standardisation and transparency in metadata capture and exchange such as the minimum information about a genome sequence (Field *et al.*, 2008), the minimum information about a marker gene sequence (Yilmaz *et al.*, 2010) or the genomic standards consortium: bringing standards to life for microbial ecology (Yimaz *et al.*, 2011). As protocols continue to evolve and diversify, guidance modules for reporting in a standardised manner, the use of techniques have also been described. Thus, the lack of consensus on how to perform qPCR experiments has led Bustin *et al.* (2009) to propose the minimum information for publication of quantitative real-time PCR experiments (MIQE) guidelines. There are several precedents such as the minimum

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information about a proteomics experiment (Taylor *et al.*, 2007) or the minimum information about a microarray experiment (MIAME) (Brazma *et al.*, 2001). The MIAME is now an accepted reference as the reflected by the number of citations, which exceed 1600 (ISI Web of Knowledge). These efforts also highlight that there are other paths for standardisation than the ISO. However, standardisation should proceed within the auspices of international working bodies and be preferably in open access or with a very low cost to facilitate the dissemination within the scientific community. Standard adoption also requires both information and a stronger involvement of leading researchers within the field. There is a clearly a need and room for new standards in soil microbiology. New standards would be beneficial to researchers, non-governmental organisations, governments, farmers and other land managers, for better monitoring soil quality and understanding of soil functioning. Developing standard protocols in soil microbiology is crucial to meet the objectives of the Millennium Ecosystem Assessment (2005) and of the emerging EU Soil Framework Directive (Commission of the European Community, 2006) for assessing the consequences of the intensification of human activities on the functioning of the soil ecosystem and its biodiversity.

In conclusion, we argue that there is a need to avoid the perhaps inevitable procrastination in setting standards that arises from the range of issues discussed earlier, and we need to be pragmatic in getting standards accepted and implemented, with caveats duly acknowledged. There is a trade-off between the urge for perfect methods vs. standardised methods, and we believe that standardisation allowing data comparison across studies, and therefore facilitating the quest for 'unifying principles in soil ecology' as described by Fierer *et al.* (2009), is more important than describing a few specific samples 'perfectly'. The rewards from such an approach would far exceed the drawbacks.

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References

Alef K & Nannipieri P (1995) *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, London, UK.

Andersch I & Anderson JPE (1991) Influence of pesticides on nitrogen transformations in soil. *Toxicol Environ Chem* **30**: 153–158.

Anderson JPE (1982) Soil respiration. *Methods of Soil Analysis, Part 2*, 2nd edn (Page A, Miller RH & Keeney DR, eds), pp. 837–871. American Society of Agronomy and Soil Science Society of America, Madison, WI, USA.

Anderson JPE & Domsch KH (1978) Physiological method for quantitative measurement of microbial biomass in soils. *Soil Biol Biochem* **10**: 215–221.

Arnebrant K & Schnurer J (1990) Changes in ATP content during and after chloroform fumigation. *Soil Biol Biochem* **22**: 875–877.

Attaway HH, Paynter MJB & Camper ND (1982) Degradation of selected phenylurea herbicides by anaerobic pond sediment. *J Environ Sci Health B* **17**: 683–699.

Beck T, Joergensen RG, Kandeler E, Makeschin F, Nuss E, Oberholzer H & Scheu S (1997) An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. *Soil Biol Biochem* **29**: 1023–1032.

Beland FA, Farwell SO & Geer RD (1974) Anaerobic degradation of 1,1,1,2-tetrachloro-2,2-bis(P-chlorophenyl) ethane(DTE). *J Agric Food Chem* **22**: 1148–1149.

Belser L & Mays E (1980) Specific inhibtion of nitrite oxidation by chlorate and its use in assessing nitrification in soil and sediment. *Appl Environ Microbiol* **39**: 505–510.

Blight EG & Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**: 911–917.

Bodelier P (2011) Towards understanding, managing and protecting microbial ecosystems. *Front Microbiol* **2**: 1–8.

Brazma A, Hingamp P, Quackenbush J *et al.* (2001) Minimum information about a microarray experiment (MIAME) – toward standards for microarray data. *Nat Genet* **29**: 365–371.

Bremner JM (1965) Nitrogen availability indexes. *Methods of Soil Analysis, Part 2* (Black CA, ed), pp. 1324–1345. American Society of Agronomy, Madison, WI.

Brookes PC, Landman A, Pruden G & Jenkinson DS (1985) Chloroform fumigation and the release of soil-nitrogen - a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol Biochem* **17**: 837–842.

Bustin SA, Benes V, Garson JA *et al.* (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* **55**: 611–622.

Chander K & Brookes PC (1991) Effects of heavy-metals from past applications of sewage-sludge on microbial biomass and organic-matter accumulation in a sandy loam and silty loam UK soil. *Soil Biol Biochem* **23**: 927–932.

Clayden B & Hollis J (1984) Critieria for differentiating soil series. *Soil Survey Technical Monograph*, Vol. 17. Rothamsted Experimental Station, Harpenden, UK, pp. 159.

Commission of the European Community (2006) Directive of the European parlament and of the council establishing a

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framework for the protection of soil and amending directive 2004/35/EC.

Creamer RE, Bellamy P, Black HIJ *et al.* (2009) An inter-laboratory comparison of multi-enzyme and multiple substrate-induced respiration assays to assess method consistency in soil monitoring. *Biol Fertil Soil* **45**: 623–633.

Delmont TO, Robe P, Cecillon S, Clark IM, Constancias F, Simonet P, Hirsch PR & Vogel TM (2011) Accessing the soil metagenome for studies of microbial diversity. *Appl Environ Microbiol* **77**: 1315–1324.

Falkowski PG, Fenchel T & Delong EF (2008) The microbial engines that drive Earth's biogeochemical cycles. *Science* **320**: 1034–1039.

FAO (2006) *World Reference Base for Soil Resources Reports No. 130*. FAO, Rome.

Feinstein LM, Sul WJ & Blackwood CB (2009) Assessment of bias associated with incomplete extraction of microbial DNA from soil. *Appl Environ Microbiol* **75**: 5428–5433.

Field D, Garrity G, Gray T *et al.* (2008) The minimum information about a genome sequence (MIGS) specification. *Nat Biotechnol* **26**: 541–547.

Fierer N, Grandy A, Six J & Paul E (2009) Searching for unifying principles in soil ecology. *Soil Biol Biochem* **41**: 2249–2256.

Findlay RH, Trexler MB, Guckert JB & White DC (1990) Laboratory study of disturbance in marine-sediments – response of a microbial community. *Mar Ecol Prog Ser* **62**: 121–133.

Frostegård Å & Bååth E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol Fertil Soil* **22**: 59–65.

Frostegård Å, Tunlid A & Bååth E (1991) Microbial biomass measured as total lipid phosphate in soils of different organic content. *J Microbiol Methods* **14**: 151–163.

Frostegård Å, Courtois S, Ramisse V *et al.* (1999) Quantification of bias related to the extraction of DNA directly from soils. *Appl Environ Microbiol* **65**: 5409–5420.

Frostegård Å, Tunlid A & Bååth E (2011) Use and misuse of PLFA measurements in soils. *Soil Biol Biochem* **43**: 1621–1625.

Fuchs M, Koch C & Wilke BM (1994) Modification of the determination of dehydrogenase activity with tetrazolium chloride for heavy metal contaminated soils). *VDLUFA Schriftenreihe* **38**: 899–902.

Gattinger A, Günthner A, Schloter M & Munch J (2003) Characterization of Archaea in soils by polar lipid analysis. *Acta Biotechnol* **23**: 21–28.

German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL & Allison SD (2011) Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biol Biochem* **43**: 1387–1397.

Glathe H & Thalmann A (1970) The microbial activity and its relationship to fertility characteristics of various farm lands with special reference to the dehydrogenase activity (TTC reduction). 2. Determination of the TTC reduction in soil in laboratory trials. *Zentralbl Bakteriol Parasitenkd Infektionskr Hyg* **124**: 24–36.

Gowda TKS & Sethunathan N (1976) Persistence of endrin in Indian rice soils under flooded conditions. *J Agric Food Chem* **24**: 750–753.

Gupta SR & Singh JS (1977) Effect of alkali concentration, volume and absorption area on measurement of soil respiration in a tropical sward. *Pedobiologia* **17**: 233–239.

Hansson G-B, Klemedtsson L, Stenström J & Torstensson L (1991) Testing the influence of chemicals on soil autotrophic ammonium oxidation. *Environ Toxicol Water Qual* **6**: 351–360.

Harden T, Joergensen RG, Meyer B & Wolters V (1993a) Mineralization of straw and formation of soil microbial biomass in a soil treated with simazine and dinoterb. *Soil Biol Biochem* **25**: 1273–1276.

Harden T, Joergensen RG, Meyer B & Wolters V (1993b) Soil microbial biomass estimated by fumigation extraction and substrate-induced respiration in 2 pesticide-treated soils. *Soil Biol Biochem* **25**: 679–683.

Hardy R, Holsten R, Jackson E & Burns R (1968) The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. *Plant Physiol* **43**: 1185–1207.

Healy JB & Young LY (1979) Anaerobic biodegradation of 11 aromatic-compounds to methane. *Appl Environ Microbiol* **38**: 84–89.

Henriksen A & Selmer-Olsen AR (1970) Automatic methods for determining nitrate and nitrite in water and soil extracts. *Analyst* **95**: 514–518.

Inubushi K, Brookes PC & Jenkinson DS (1991) Soil microbial biomass C, N and ninhydrin-N in aerobic and anaerobic soils measured by the fumigation-extraction method. *Soil Biol Biochem* **23**: 737–741.

ISO/IEC (2004) *Guide 2 – Standardization and Related Activities – General Vocabulary*. International Organization for Standardization, Geneva, Switzerland, pp. 60.

ISO/IEC (2009) *ISO/IEC Directives, Part 1 Procedures for the Technical Work*, 7th edn. International Organization for Standardization, Geneva, Switzerland, pp. 80.

Jenkinson DS & Powlson DS (1976) Effects of biocidal treatments on metabolism in soil. 5. Method for measuring soil biomass. *Soil Biol Biochem* **8**: 209–213.

Kaur A, Chaudhary A, Kaur A, Choudhary R & Kaushik R (2005) Phospholipid fatty acid – a bioindicator of environment monitoring and assessment in soil ecosystem. *Curr Sci* **89**: 1103–1112.

Kearney PC (1982) IUPAC pesticide commission report. *J Assoc Off Anal Chem* **65**: 1030–1032.

Krsek M & Wellington EMH (1999) Comparison of different methods for the isolation and purification of total community DNA from soil. *J Microbiol Methods* **39**: 1–16.

deLiphtay JR, Enzinger C, Johnsen K, Aamand J & Soerensen SJ (2004) Impact of DNA extraction method on bacterial community composition measured by denaturing gradient gel electrophoresis. *Soil Biol Biochem* **35**: 1607–1614.

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Martin-Laurent F, Philippot L, Hallet S, Chaussod R, Germon JC, Soulas G & Catroux G (2001) DNA extraction from soils: old bias for new microbial diversity analysis methods. *Appl Environ Microbiol* **67**: 2354–2359.

Marx MC, Wood M & Jarvis SC (2001) A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biol Biochem* **33**: 1633–1640.

Marx MC, Kandeler E, Wood M, Wermbter N & Jarvis SC (2005) Exploring the enzymatic landscape: distribution and kinetics of hydrolytic enzymes in soil particle-size fractions. *Soil Biol Biochem* **37**: 35–48.

vonMersi W & Schinner F (1991) An improved and accurate method for determining the dehydrogenase-activity of soils with iodonitrotetrazolium chloride. *Biol Fertil Soil* **11**: 216–220.

Millennium Ecosystem Assessment (2005) *Ecosystems and Human Well-Being: Biodiversity Synthesis*. World Resources Institute, Washington, DC.

Mueller T, Joergensen RG & Meyer B (1992) Estimation of soil microbial biomass-C in the presence of living roots by fumigation extraction. *Soil Biol Biochem* **24**: 179–181.

Nelson D & Sommers L (1996) Total carbon, organic carbon and organic matter. *Methods of Soil Analysis Part 3: Chemical Methods* (Sparks D, ed), pp. 961–1010. Soil Science Society America, Madison, WA.

Niemi RM & Vepsalainen M (2005) Stability of the fluorogenic enzyme substrates and pH optima of enzyme activities in different Finnish soils. *J Microbiol Methods* **60**: 195–205.

Niemi RM, Heiskanen I, Wallenius K & Lindstrom K (2001) Extraction and purification of DNA in rhizosphere soil samples for PCR-DGGE analysis of bacterial consortia. *J Microbiol Methods* **45**: 155–165.

Nordgren A (1988) Apparatus for the continuous, long-term monitoring of soil respiration rate in large numbers of samples. *Soil Biol Biochem* **20**: 955–957.

Nordgren A, Baath E & Soderstrom B (1988) Evaluation of soil respiration characteristics to assess heavy-metal effects on soil-microorganisms using glutamic-acid as a substrate. *Soil Biol Biochem* **20**: 949–954.

Nortcliff S (2002) Standardisation of soil quality attributes. *Agric Ecosyst Environ* **88**: 161–168.

Ocio JA & Brookes PC (1990) An evaluation of methods for measuring the microbial biomass in soils following recent additions of wheat straw and the characterization of the biomass that develops. *Soil Biol Biochem* **22**: 685–694.

Ohlinger R (1995) Determination of dehydrogenase activity using TTC. *Methods in Soil Biology* (Schinner F, Ohlinger R, Kandeler E & Margesin R, eds), pp. 426. Springer-Verlag, Berlin, Germany.

Olsson PA (1999) Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *FEMS Microbiol Ecol* **29**: 303–310.

Pan Y, Bodrossy L, Frenzel P *et al.* (2010) Impact of inter- and intralaboratory variation on the reproducibility of microbial community analyses. *Appl Environ Microbiol* **76**: 7451–7458.

Petric I, Philippot L, Abbate C *et al.* (2011) Inter-laboratory evaluation of the ISO standard 11063 "Soil quality – Method to directly extract DNA from soil samples". *J Microbiol Methods* **84**: 454–460.

Ritz K (2008) Soil as a paradigm of a complex system. *Complexity and Security* (Ramsden JJ & Kervalishvili PJ, eds), pp. 103–119. NATO Science for Peace and Security Series: Human and Societal Dynamics (Volume 37). IOS Press, Amsterdam. ISBN: 978-1-58603-849-6.

Selmer-Olsen AR (1971) Determination of ammonium in soil extracts by an automated indophenol method. *Analyst* **96**: 565–568.

Shelton DR & Tiedje JM (1984) General-method for determining anaerobic biodegradation potential. *Appl Environ Microbiol* **47**: 850–857.

Smalla K, Cresswell N, Mendoncahagler LC, Wolters A & VanElsas JD (1993) Rapid DNA extraction protocol from soil for polymerase chain reaction-mediated amplification. *J Appl Bacteriol* **74**: 78–85.

Smith M & Tiedje JM (1979) Phases of denitrification following oxygen depletion in soil. *Soil Biol Biochem* **11**: 261–267.

Sparling GP, Feltham CW, Reynolds J, West AW & Singleton P (1990) Estimation of soil microbial C by a fumigation extraction method - use on soils of high organic-matter content, and a reassessment of the K_{ec}-factor. *Soil Biol Biochem* **22**: 301–307.

Spothelfer-Magaña J, Thalmann A & Schweikle V (1993) Methode zur bestimmung der dehydrogenaseaktivität von böden unter einsatz von Iodonitrotetrazolium-chlorid (INT): Chemische reduktion in autoklavierten sowie bestrahlten böden und der einfluß der inkubationstemperatur und -zeit. *Agribiol Res* **46**: 250–268.

Stanford G & Smith SJ (1972) Nitrogen mineralization potentials of soils. *Soil Sci Soc Am J* **36**: 465–472.

Stemmer M, Gerzabek MH & Kandeler E (1998) Organic matter and enzyme activity in particle-size fractions of soils obtained after low-energy sonication. *Soil Biol Biochem* **30**: 9–17.

Stenberg B, Johansson M, Pell M, Sjödahl-Svensson K, Stenström J & Torstensson L (1998) Microbial biomass and activities in soil as affected by frozen and cold storage. *Soil Biol Biochem* **30**: 393–402.

Stenstrom J, Stenberg B & Johansson M (1998) Kinetics of substrate-induced respiration (SIR): theory. *Ambio* **27**: 35–39.

Tabatabai MA (1994) *Soil enzymes. Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties* (Weaver RW, Angle S, Bottomley P, Bezdicek D, Smith S, Tabatabai MA & Wollum A, eds), pp. 775–833. Soil Science Society of America, Madison.

Taylor CF, Paton NW, Lilley KS *et al.* (2007) The minimum information about a proteomics experiment (MIAPE). *Nat Biotechnol* **25**: 887–893.

Thalmann A (1968) Zur methodik der bestimmung der dehydrogenaseaktivität im boden mittels

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Triphenyltetrazoliumchlorid (TTC). *Landwirtsch Forsch* **21**: 249–258.
Torsvik V, Øvreås L & Thingstad TF (2002) Prokaryotic diversity – magnitude, dynamics, and controlling factors. *Science* **296**: 1064–1066.
Tsai YL & Olson BH (1991) Rapid method for direct extraction of DNA from soil and sediments. *Appl Environ Microbiol* **57**: 1070–1074.
van der Heijden MGA, Bardgett RD & van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett* **11**: 296–310.
van Elsas JD, Smalla K & Tebbe CC (2000) Extraction and analysis of microbial community nucleic acids from environmental matrices. *Tracking Genetically Engineered Microorganisms* (Jansson JK, van Elsas JD & Bailey MJ, eds), pp. 29–61. Landes Bioscience, Georgetown, TX.
VanBeelen P, Fleuren-Kemila AK, Huys MPA, VanMontfort ACP & VanVlaardingen PLA (1991) The toxic effects of pollutants on the mineralization of acetate in subsoil microcosms. *Environ Toxicol Chem* **10**: 775–789.
Vance ED, Brookes PC & Jenkinson DS (1987) An extraction method for measuring soil microbial biomass-C. *Soil Biol Biochem* **19**: 703–707.
Vepsäläinen M, Kukkonen S, Vestberg M, Sirvio H & Niemi RM (2001) Application of soil enzyme activity test kit in a field experiment. *Soil Biol Biochem* **33**: 1665–1672.
Vepsäläinen M, Erkomaa K, Kukkonen S, Vestberg M, Wallenius K & Niemi RM (2004) The impact of crop plant cultivation and peat amendment on soil microbial activity and structure. *Plant Soil* **264**: 273–286.
Vogel TM, Simonet P, Jansson JK *et al.* (2009) TerraGenome: a consortium for the sequencing of a soil metagenome. *Nat Rev Microbiol* **7**: 252.
Wallenstein M & Weintraub M (2008) Emerging tools for measuring and modeling the *in situ* activity of soil extracellular enzymes. *Soil Biol Biochem* **40**: 2098–2106.
Ward TE (1986) Aerobic and anaerobic biodegradation of nitrilotriacetate in subsurface soils. *Ecotoxicol Environ Saf* **11**: 112–125.
Watts CW, Eich S & Dexter AR (2000) Effects of mechanical energy inputs on soil respiration at the aggregate and field scales. *Soil Till Res* **53**: 231–243.
Wessen E & Hallin S (2011) Abundance of archaeal and bacterial ammonia oxidizers – possible bioindicator for soil monitoring. *Ecol Indic* **11**: 1696–1698.
White DC, Davis WM, Nickels JS, King JD & Bobbie RJ (1979) Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia* **40**: 51–62.
Whitman WB, Coleman DC & Wiebe WJ (1998) Prokaryotes: the unseen majority. *P Natl Acad Sci USA* **95**: 6578–6583.
Wilke BM (1982) Lead sorption and effect of lead pollution on biological-activity of different types of humus forms. *Z Pflanzen Bodenk* **145**: 52–65.
Wilke BM, Winkel B, Fleischmann S & Gong P (1998) *Higher Plant Growth and Microbial Toxicity Tests for the Evaluation of the Ecotoxic Potential of Soils*. Thomas Telford Ltd, London, pp. 345–354.
Winkel B, Saeger T & Wilke B-M (1999) Bewertung kontaminierter Böden mit Hilfe von potentieller Nitrifikation. *Ökotoxicologie-Ökosystemare Ansätze und methoden* (Oehlmann J & Markert B, eds), pp. 67–72. ECOMED Verlag.
Wu J, Joergensen RG, Pommerening B, Chaussod R & Brookes PC (1990) Measurement of soil microbial biomass C by fumigation extraction – an automated procedure. *Soil Biol Biochem* **22**: 1167–1169.
Yilmaz P, Kottmann R, Field D *et al.* (2010) Minimum information about a marker gene sequence (MIMARKS) and minimum information about any (x) sequence (MIxS) specifications. *Nat Biotechnol* **29**: 415–420.
Yimaz P, Gilbert JA, Kniht R, Amaral-Zettler L, Karsh-Mizachi I, Cochrane G, Nakamura Y, Sansone SA, Glockner FO & Field D (2011) The genomic standards consortium: bringing standards to life for microbial ecology. *ISME J* **5**: 1565–1567.
Zelles L (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biol Fertil Soil* **29**: 111–129.
Zelles L & Bai QY (1993) Fractionation of fatty-acids derived from soil lipids by solid-phase extraction and their quantitative-analysis by GC-MS. *Soil Biol Biochem* **25**: 495–507.
Zhou JZ, Bruns MA & Tiedje JM (1996) DNA recovery from soils of diverse composition. *Appl Environ Microbiol* **62**: 316–322.

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