Journal of Microbiological Methods 165 (2019) 105695

Contents lists available at ScienceDirect

Journal of Microbiological Methods

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

Coring lubricants can increase soil microbial activity in Vertisols

Katherine Polain[a,*], Gabrielle Joice[b], Dominic Jones[c], Lily Pereg[a], Gunasekhar Nachimuthu[d], Oliver G.G. Knox[a]

[a] Faculty of Science, Agriculture, Business and Law, University of New England, Armidale, NSW 2350, Australia
[b] McCarthy Catholic College, North Tamworth, NSW 2340, Australia
[c] Oxley High School, Tamworth, NSW 2340, Australia
[d] NSW DPI, Locked bag 1000, Narrabri, NSW 2390, Australia

ARTICLE INFO

Keywords:
Vertosol
Soil sampling
Biological indicators
Soil microbial analysis
Substrate induced respiration

ABSTRACT

It is essential that sampling procedures for biological measurements are done in a way that reflects the soil processes, whilst limiting sampling artefacts. In heavy clay Vertisol soils, coring lubricants are often considered necessary in order to extract and recover soil for quality and health assessments. Previous reports into the use of coring lubricants have found soil carbon measurements to be inflated but to date, a study to evaluate the effects of these lubricants on soil microbial activity, has not been forthcoming. We measured soil carbon dioxide (CO_2) evolution in response to the addition of common coring lubricants, to determine the effects upon soil microbial activity to the depth of 100 cm. Application of coring lubricants to the surface soil layers of field collected cores did not significantly influence CO_2 evolution however, microbial activity increased in deeper soil layers (30–100 cm) with the use of WD-40, mould stripper and silicone oil. When the ratio of coring lubricant to soil was increased to ~5 g coring lubricant to 100 g^{-1} soil, there was a significant ($P = .001$) effect on microbial activity, with silicone oil and mould stripper inflating measurements by at least 5%, whilst olive oil and WD-40 were similar to the control. The results imply that when using coring rigs to recover soil for microbial functional analysis in Vertisols, the use of coring lubricants is best avoided, with further research recommended.

1. Introduction

Productive farming requires a delicate balance between profitability, necessary inputs and sustainability of the whole system (Doran, 2002; Barrios, 2007). This balance needs to be achieved to support an increasing global population whilst countering the effects of climate change and a history of intensive agriculture, resulting in a depletion of soil resources (Schloter et al., 2018). Therefore, the monitoring of soil health and quality to inform farm management decisions is a critical component of sustainable farming practice (Doran, 2002; Schloter et al., 2018). Biological soil quality indicators, such a soil microbial activity, are acknowledged as some of the most under-utilised monitoring tools, despite being crucial for assessing land management and climate change effects on soil function (Doran, 2002; Nielsen et al., 2002; Schloter et al., 2003; Bünemann et al., 2018).

Soil microorganisms are responsible for the cycling of carbon (C) and key plant nutrients such as nitrogen (N) and phosphorus (P). Consequently, they are the best biological indicators of cause and effect relationships between agricultural practice and the eventual productivity of soils (Barrios, 2007), as a result of their rapid response to their environment (Doran and Zeiss, 2000; Nielsen et al., 2002; Schloter et al., 2003). However, field assessment of microbial function and activity can be limited (Pell et al., 2007) and it is therefore important to collect soil samples for laboratory analysis in a manner that does not compromise subsequent measurements.

Coring in heavy clay soils (30–95% clay content), such as Vertisols (Pierre et al., 2019; Williams et al., 1983), is often challenging, with modifications to coring tubes necessary to suit different moisture conditions. However, the application of a lubricant to prevent soil from sticking to the inside of the coring tube and compaction whilst pushing the soil cores out of the tubes, is a neccessary practice in heavy clays (Gregorich and Carter, 2007). Increasing concerns relating to erroneous soil organic carbon (SOC) measurements due to C contamination in coring lubricants have been investigated (Orgill et al., 2015; Dowling et al., 1985). Dowling et al. (1985) found that commonly used mould oil (shuttering oil) lubricant interfered with SOC measurements, with a mean maximum increase of 3.17% C in the lubricant contaminated soil. Yet, the effects (i.e. inhibition or stimulation) of these coring lubricants on soil microbial activity is yet to be investigated.

Soil microorganisms are potentially exposed to coring lubricants on

* Corresponding author.
E-mail address: kpolain2@une.edu.au (K. Polain).

https://doi.org/10.1016/j.mimet.2019.105695
Received 26 June 2019; Accepted 19 August 2019
Available online 20 August 2019

the inner surface of the coring tube through sampling and homogenisation of the soil samples, where the latter increases the ratio of soil surface to coring lubricant coverage. Therefore, we conducted two substrate induced respiration (SIR) experiments to determine the effect of coring lubricants on soil microbial activity. Soil cores were recovered from 0 to 100 cm on a Vertisol, in the northern tablelands of New South Wales (NSW), Australia. Vertisols are clay-rich and become prone to adhering to the coring tube when wet, making core extraction without the use of coring lubricants challenging. The effect of coring lubricants on the microbial activity of these soils is of particular interest to the Australian cotton industry, where regular monitoring of soil health and quality, commonly include the use of coring lubricants such as plant oils, silicone oils, mould stripper and synthetics (i.e. WD-40). This study was designed to determine if a decision not to use coring lubricants in a wider microbial interrogation of the Australian cotton soils (the majority of which are Vertisols) (Hulugalle and Scott, 2008), was valid. This study is first of its kind, attempting to bridge the knowledge gap relating the influence of coring tube lubricants on soil microbial activity measurements.

2. Material and methods

2.1. Experimental soil sampling

Five soil cores were collected from the 'Trevenna' farm at the University of New England, NSW (Australia) (Latitude 30°29′12.21″S, Longitude 151°38′0.90″E). Each core was recovered from a depth of 100 cm using a powered soil corer with a 40 mm internal diameter polyvinyl chloride (PVC) half-pipe insert. The insert stored and supported the cores, which were sealed at the ends with plastic wrap and transported back to the laboratory in a chilled box for processing on the same day as recovery and within four hours of the first core being extracted.

2.2. Soil properties

The soils were classified as Black-grey Vertosol (Isbell, 2016) or Vertisol (IUSS Working Group, 2006). Three additional cores were taken for physical and chemical analysis (Table 1). The majority of analyses were undertaken by CSBP Soil and Plant Analysis Laboratory (Perth, Western Australia), on composite samples taken from three independent cores that were subdivided into the respective depths (10–30, 30–50, 50–70 and 70–100 cm) (for method details, see CSBP (2019)). Exceptions to this were particle size analysis (PSA), which was conducted at UNE following the methods of McDonald et al. (1984) and dissolved organic carbon (DOC), which was analysed on a InnovOx TOC analyser (GE Analytical Instruments) from a 1 in 4 soil to 5 mM $CaCl_2$ extraction. The cores were assumed to be similar based on ANOVA of the DOC and PSA undertaken on each depth of these three cores, which returned P values of 0.45 and 1, respectively. DOC differed by depth ($P < .001$), with 10–30 cm being higher than all other depths (30–100 cm) which were statistically similar. The PSA did not differ by depth ($P = .914$). The gravimetric water content (GWC) at the time of sampling was not significantly different according to soil layer ($P = .22$), and was 20% in the 10–30 cm soil layer, 36% in both the 30–50 and 50–70 cm layers and 30% in the 70–100 cm layer.

2.3. Core partitioning and respiration set up

Each 100 cm core was partitioned into 4 depths (10–30, 30–50, 50–70 and 70–100 cm) for analysis. The 0–10 cm fraction was not included due to exposure to large diurnal and seasonal fluctuations in temperature and moisture, which generally result in a low microbial biomass and a lack of structural integrity, making handling difficult. From each depth partition, four sub-cores measuring 40 mm (diameter) by 30 mm (length) were cut and weighed in preparation for respiration incubations. The first experiment was conducted by exposing the circumferential surface of these intact soil cores to common coring lubricants and measuring CO_2 evolution using a Respicond respirometer (Nordgren, 1988). The remaining soil at each depth was sampled using a sterile cork borer to produce an additional four mini sub-cores measuring 4 mm (diameter) by 20 mm (length), which were transferred to a 1.2 mL 96 deep well plate for a second experiment involving increased soil exposure to the coring lubricants with CO_2 evolution determined by MicroResp (Campbell et al., 2003).

The recovered 30 mm by 40 mm cores were designated at random as either a control (water) or exposed to one of either silicone oil (bulk density 1.00 g/cm^3, 33% C), mould stripper (bulk density 0.92 g/cm^3, 40% C) or WD-40 (bulk density 0.88 g/cm^3, 44% C) coring lubricants. The exposure to the coring lubricant was conducted by spraying each lubricant onto the surface of a 100 × 200 mm plastic sheet, which was then weighed. An intact core was placed onto the plastic sheet and rolled, using the sheet to apply the lubricant to the core outer surface to mimic normal field soil coring and lubricant exposure conditions. The core was then inserted into a respirometer pot (Nordgren, 1988) and the amount of lubricant applied was determined by reweighing the plastic sheet and calculating the difference in the weight of the lubricant on the plastics prior to and after rolling of the soil core. The pots were loaded into a 20 °C water bath and incubated for 48 h. Carbon dioxide evolution was determined by a decrease in conductivity, which was calculated automatically and converted to either hourly CO_2 or cumulative CO_2 by the Respicond software (Nordgren, 1988).

To the 4 mm by 20 mm cores in the 96 well plate, 20 µL of either water (control), glucose solution (100 g L^{-1}) (positive control), silicone oil, WD-40, mould stripper or olive oil (bulk density 0.84 g/cm^3, 78% C) was added. These cores were incubated in a MicroResp system (Campbell et al., 2003) for 24 h. The respired CO_2 was captured in a detection plate and the colour change was measured on a SpectraMax M2e multi-mode plate reader (Molecular Devices Corporation) at 570 nm, with all data handling performed according to the MicroResp user guide (Campbell et al., 2003).

2.4. Statistical analysis

All data was tabulated in Microsoft Excel and imported into the GenStat program (VSN International Limited, U.K.) for statistical analyses. Where sample size was considered small, paired t-test was used to assess significances between measures. For all other inquiries, Analysis

Table 1
Characteristics of the Trevena Vertisol soil from composite samples analysed by CSPB and depth sub-samples of three cores analysed at UNE. Similar means ($P < .001$), based on ANOVA of 12 samples are indicated by the same lower case letter.

Depth (cm)	Gravel	Sand	Silt	Clay	Ammonium	Nitrate	Colwell P	PBI	Colwell K	Sulfur	OC	DOC	Conductivity	ESP	pH
	%	%	%	%	mg/kg	mg/kg	mg/kg		mg/kg	mg/kg	%	ug/g	dS/m	%	($CaCl_2$)
10–30	0	21	25	55	3	< 1	13	139.0	220	1.9	1.81	12.3[a]	0.031	1.7	5.2
30–50	0	20	23	57	3	< 1	7	169.9	183	1.2	0.89	7.4[b]	0.028	2.6	5.8
50–70	0	21	22	57	3	< 1	4	166.1	172	0.8	0.58	6.9[b]	0.036	3.2	6.3
70–100	0	27	28	45	1	< 1	5	167.4	170	0.6	0.41	9.1[b]	0.036	3.0	6.8

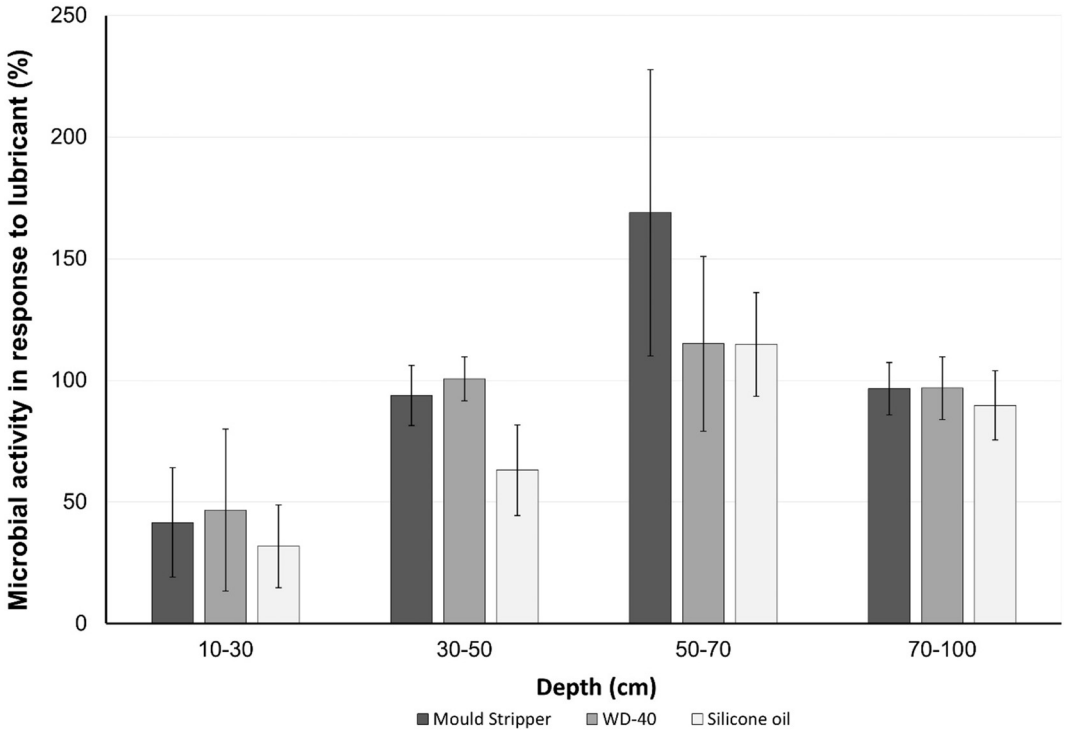

Fig. 1. The effect of mean coring lubricant addition, as a percentage (%) of the control on microbial activity as measured by average hourly microbial activity over 48 h. A value < 100% indicates inhibited microbial activity, whilst values above 100% indicate increased microbial activity (n = 4).

of Variance (ANOVA) was used to assess significant differences ($P \leq .05$) between the experimental factors of depth, lubricant and lubricant by depth, with blocking by cores.

3. Results

3.1. Core circumference oil application and respiration

Exposure of the circumferential surface of intact soil cores to common coring lubricants found the adherence of silicone oil (0.36 g lubricant 100 g^{-1} soil) to the soil cores to be significant ($P < .001$) and three times higher than either WD-40 or mould stripper (0.12 and 0.11 g lubricant 100 g^{-1} soil, respectively). There was no significant difference in the cumulative CO_2 after 48 h respiration between any of the experimental variants (depth, lubricant or lubricant by depth). However, the average hourly CO_2 showed a significant difference ($P = .031$) in treatment by depth, driven by increased CO_2 evolution in the top soil (10–30 cm) of the control samples. When we compared microbial activity to depth as a percentage of the control (Fig. 1), there was a trend of inflated microbial activity with increasing depth to 50–70 cm in response to all lubricants, particularly mould stripper**.** A comparison between the assumed available C in the system (DOC and coring lubricant) and the quantity of C respired at each depth, was < 1% for all coring lubricants, with no significant differences.

3.2. Lubricant effect on mini sub-cores

The 4 mm by 20 mm cores were exposed to approximately ~5 g lubricant 100 g^{-1} soil, where there was a significant difference in microbial activity by lubricant ($P = .001$, Fig. 2) and by depth ($P = .004$), but there was no apparent lubricant by depth interaction ($P = .98$). Overall, microbial activity measurements were inflated by 6% in response to the application of silicone oil and mould stripper (Fig. 2), whilst olive oil and WD-40 had no discernible effect on the respiration activity measured. Although there was no interaction between depth and lubricant, respiration was significantly higher in the 30–50 and 50–70 cm soils than in the 10–30 and 70–100 cm soils, which were statistically similar. In alignment with the circumferential data, comparison between the assumed available C in the system and the quantity of C respired at each depth, was < 1% for all coring lubricants, with no significant differences.

4. Discussion

4.1. Relationship between lubricants and carbon

The potential for coring lubricants to impact on soil C determination as a result of contamination from their use in soil coring has recently gained some interest (Orgill et al., 2015). Analysis of the C content of the oils used in this experiment highlights that whilst similarly named oils may be applied as a lubricant, they can vary in C composition. For example Orgill et al. (2015) reported of 32% C for silicone oil and 75% for mould stripper, whilst those we used were 33% and 40%, respectively. Based on the adherence of the coring lubricants and their % C, there is the potential for a significant increase in C measurements of between 2.4 and 28.3% of the existing soil C (data not shown), depending on the soil depth and coring lubricant used. In general, the % change in C due to lubricant addition increased with depth (3.7, 8.6, 13.8 and 17.0% from surface to 100 cm, respectively), as soil C concentration decreased, and was significantly higher with silicone oil ($P < .001$) compared with the other coring lubricants used (6.7, 7.8 and 17.8% increase in soil C for mould stripper, WD-40 and Silicon oil, respectively). In most cases, the change in soil C concentration may well be indiscernible within normal instrument limitations.

4.2. Effect of core circumference lubricant application on microbial activity

Whilst the influence of coring lubricants on soil C is of interest, the potential for these lubricants to alter soil microbial activity has received little to no attention. This may, in part, be due to the hydrocarbon content of coring lubricants being considered as recalcitrant to most microorganisms, with only some species able to metabolise these C

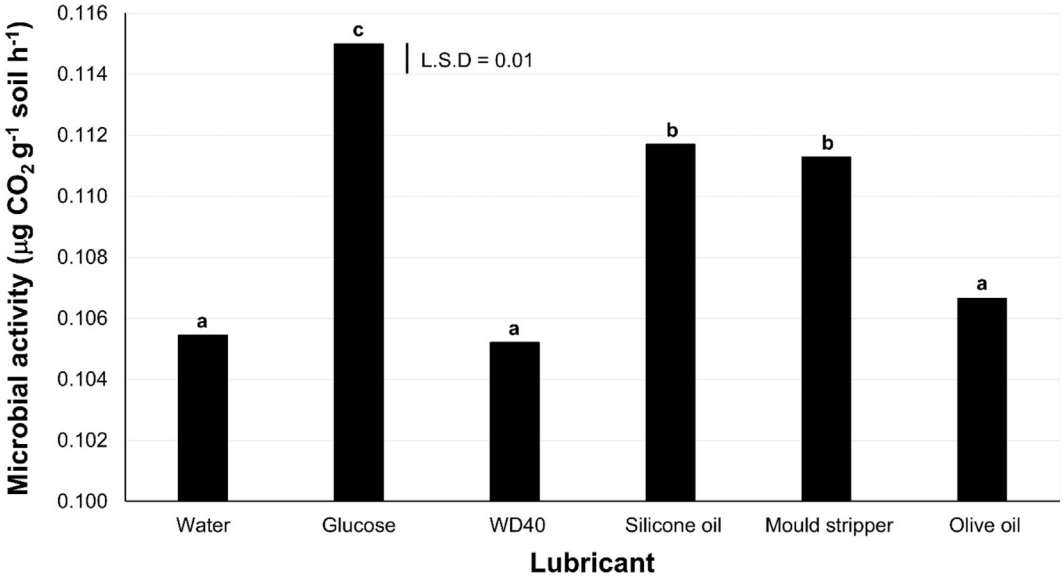

Fig. 2. Mean respiration rates (µg CO_2 g^{-1} soil h^{-1}) calculated for soil samples from 10 to 100 cm (based on no depth interaction) for coring lubricants (WD-40, Silicone oil, Mould stripper oil, and Olive oil), water (baseline control) and glucose (microbial responsiveness control). Similar alphabetical numbers represent similar means based on ANOVA and $P < .05$ and least significant difference (L.S.D) indicated by the bar and value of 0.01.

forms (Speight and Arjoon, 2012). This was upheld in our observation that the respirational C loss over the course of the experiments was < 1% of the available C. This small change in metabolism also implies that priming of soil was unlikely to be occurring within these systems and that microbial responses were based on coring lubricant addition alone. The formulation of the C present in the coring lubricants also appeared important in that, despite a lower C content, the silicone oil (33% C) inflated microbial activity proportionally to the application of an equal volume of the mould stripper (40% C), whereas olive oil (78% C) was not significantly different from the control (Fig. 2). Soil microorganisms need to be in close proximity to C sources for metabolism to occur (Blagodatskaya and Kuzyakov, 2013; Joergensen and Wichern, 2018), therefore it is plausible that the increased adherence of the silicone oil to the soil, as evidenced in the uptake from the plastic film (core circumferential application experiment), led to increased C metabolism.

In our experiments, circumferential application of coring lubricants did not significantly influence either the rate or cumulative respiration activity of microbes in the soil cores over 48 h. However, there was a trend of increased respiration as a percentage of the control in response to the application of silicone oil, WD-40 and mould stripper (Fig. 1), with activity inflation peaking at 50–70 cm. The rising microbial activity with lubricant by depth could be a function of higher GWC in the 30–70 cm region of the soil profile, possibly carrying and making C more readily available for the microorganisms to metabolise (Blagodatskaya and Kuzyakov, 2013; Demoling et al., 2007). There was evidence of a decline in soil DOC with depth (Table 1), adding the possibility for readily metabolised C in the coring lubricants to be utilised, particularly in the subsoil (30–100 cm), where microbes were more responsive to additional C sources (Fig. 1) (Joergensen and Wichern, 2018; Blagodatskaya and Kuzyakov, 2013).

4.3. Effect of high coring lubricant application on microbial activity

The MicroResp experiments indicated that increasing silicone oil and mould stripper to an excessive amounts of lubricant, compared circumferential application in normal coring operations, significantly increased microbial activity measurements (Fig. 1) compared to the control. One of the biggest challenges that this poses is in assessing what is 'normal practice'. Our observation of different coring teams at work in the field implies that this 'normal practice' is likely to range (for an example see Orgill et al., 2015). Some crews are cautious in their use of lubricants, whereas others are liberal, often only ceasing the application of the coring lubricant once the entire internal surface of the coring tube is lined with a thick, frothy coating. The significance of the microbial response to these lubricants, in conjunction with the lack of response to olive oil (a less commonly used coring lubricant) and WD-40 implies that when soil coring for microbial analysis, the choice of lubricant to limit potential inflated activity becomes important, in addition to avoiding erroneous C measurements.

4.4. Further research

Further research into how microbial activity is affected by coring lubricants is still required. Whilst our experiment was limited to one soil type, it is recognised that soils with high clay content are more adhesive to coring and other farming equipment (IUSS Working Group, 2006), so our results are applicable for any microbial activity being measured in soils with a substantial clay content and low OC (Table 1). Although it is recommended for biological soil analyses to be performed immediately after sampling, as we have done, the reality is that this is not always possible. Therefore, the effects of prolonged microbial exposure to coring lubricants stored field fresh, in the refrigerator or freezer, also requires further investigation. Additionally, our experimental approach was to both mimic and exceed the exposure of the soil to the coring lubricants, primarily to limit cross contamination between cores at sampling. In some instances, it may be possible to wash coring tubes between samples, although the practicality of such activities may well make this approach infeasible. However, if either sufficient coring tubes were available or if coring lubricant cross contamination could be eliminated, then the actual impact of these lubricants from field use would be a further avenue of research.

5. Conclusions

Our work demonstrates the need to consider how the collection of soil samples will influence the assessment of microbial activity. Our work was limited to one soil type and a narrow selection of coring lubricants. In addition, we did not capture changes in core recovery time associated with operator decisions around the use of coring lubricants.

However, given the findings of our experiments, investigations into these aspects of soil core recovery would be a logical progression. What was apparent from our experiments was that the use of common coring lubricants containing silicone or mould stripper can potentially inflate microbial activity analyses. The increasing interest in subsoil microbiological properties (Polain et al., 2018; Van Leeuwen et al., 2017), necessitates further research into the effects of coring lubricants, as biological indicators are important measurements when assessing overall soil health and quality. Therefore it is important that the data truly reflects the processes occurring in soil to inform productive farming practice in the face of global challenges.

Funding details

This work was supported by the Cotton Research and Development Corporation (CRDC) under grant UNE1601 and in collaboration with the UNE Growing Regional Agricultural and Science Students (GRASS) Programme.

Declaration of Competing Interest

The authors declare that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

References

Barrios, E., 2007. Soil biota, ecosystem services and land productivity. Ecol. Econ. 64, 269–285.
Blagodatskaya, E., Kuzyakov, Y., 2013. Active microorganisms in soil: critical review of estimation criteria and approaches. Soil Biol. Biochem. 67, 192–211.
Bünemann, E.K., Bongiorno, G., Bai, Z., Creamer, R.E., De Deyn, G., De Goede, R., Fleskens, L., Geissen, V., Kuyper, T.W., Mäder, P., 2018. Soil quality–a critical review. Soil Biol. Biochem. 120, 105–125.
Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Appl. Environ. Microbiol. 69, 3593–3599.
CSBP, 2019. *CSBP Lab Methods* [Online]. CSBP Soil and Plant Analysis Laboratory, Perth, Western Australia Available: csbp.com.au/csbp-lab (Accessed 2019).
Demoling, F., Figueroa, D., Bååth, E., 2007. Comparison of factors limiting bacterial growth in different soils. Soil Biol. Biochem. 39, 2485–2495.
Doran, J.W., 2002. Soil health and global sustainability: translating science into practice. Agric. Ecosyst. Environ. 88, 119–127.
Doran, J.W., Zeiss, M.R., 2000. Soil health and sustainability: managing the biotic component of soil quality. Appl. Soil Ecol. 15, 3–11.
Dowling, A., Shaw, R., Berthelsen, S., 1985. The influence on soil properties of mould oil used in sample cores. Soil Res. 23, 655–659.
Gregorich, E.G., Carter, M.R., 2007. Soil Sampling and Methods of Analysis. CRC Press.
Hulugalle, N.R., Scott, F., 2008. A review of the changes in soil quality and profitability accomplished by sowing rotation crops after cotton in Australian Vertosols from 1970 to 2006. Soil Res. 46, 173–190.
Isbell, R., 2016. The Australian Soil Classification. CSIRO Publishing.
IUSS WORKING GROUP, W, 2006. World reference base for soil resources. World Soil Resour. Rep. 103.
Joergensen, R.G., Wichern, F., 2018. Alive and kicking: why dormant soil microorganisms matter. Soil Biol. Biochem. 116, 419–430.
McDonald, R., Isbell, R., Speight, J., Walker, J., Hopkins, M., 1984. Australian soil and land survey. Field Handbook. Australian soil and land survey. Field Handbook.
Nielsen, M.N., Winding, A., Binnerup, S., 2002. Microorganisms as Indicators of Soil Health.
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.
Orgill, S., Doran, G., Conyers, M., Orchard, B., Oates, A., Poile, G., Hayes, R., Hawkins, P., Xu, B., 2015. Activity Report EverCrop Carbon Plus. CSIRO.
Pell, M., Stenstrom, J., Granhall, U., 2007. Soil respiration. In: Bloem, J., Hopkins, D.W., Benedetti, A. (Eds.), Microbiological Methods for Assessing Soil Quality. CABI.
Pierre, T.J., Primus, A.T., Simon, B.D., Hamadjida, G., Monique, A., Pierre, N.J., Lucien, B.D., 2019. Characteristics, classification and genesis of vertisols under seasonally contrasted climate in the Lake Chad Basin, Central Africa. J. Afr. Earth Sci. 150, 176–193.
Polain, K., Guppy, C., Knox, O., Lisle, L., Wilson, B., Osanai, Y., Siebers, N., 2018. Determination of agricultural impact on soil microbial activity using δ18OP HCl and respiration experiments. ACS Earth Space Chem. 2, 683–691.
Schloter, M., Dilly, O., Munch, J., 2003. Indicators for evaluating soil quality. Agric. Ecosyst. Environ. 98, 255–262.
Schloter, M., Nannipieri, P., Sørensen, S.J., Van, E.L.S.A.S., D, J., 2018. Microbial indicators for soil quality. Biol. Fertil. Soils 54, 1–10.
Speight, J.G., Arjoon, K.K., 2012. Bioremediation of Petroleum and Petroleum Products. John Wiley & Sons.
Van Leeuwen, J., Djukic, I., Bloem, J., Lehtinen, T., Hemerik, L., De Ruiter, P., Lair, G., 2017. Effects of land use on soil microbial biomass, activity and community structure at different soil depths in the Danube floodplain. Eur. J. Soil Biol. 79, 14–20.
Williams, J., Prebble, R., Williams, W., Hignett, C., 1983. The influence of texture, structure and clay mineralogy on the soil moisture characteristic. Soil Res. 21, 15–32.