Substrates used in SIR assays can inhibit basal respiration in rewetted soil

Katarzyna M. Bialkowski[A,B], Robert Archibald[A], Giles E. StJ. Hardy[A] and Treena I. Burgess[A]

[A]School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA, Australia.
[B]Corresponding author. Email K.Bialkowski@murdoch.edu.au

Abstract
Respiration assays are routinely used for investigating microbial metabolic activity in soil, but usually after a period of "conditioning" whereby dry soil is rewetted and incubated for a period of days. We showed that rewetting and incubation of soil with or without amendments cause changes in microbial populations that are dependent on the type of amendment. As these amendments resulted in altered basal respiration levels and SIR profiles, they call into question the suitability of soil conditioning as pretreatment for soil microbial analyses. When testing soils from an experiment involving various amendments we have found that different substances can inhibit, rather than stimulate, respiration following rewetting. We suggest further investigation of "CO_2 burst inhibition" for the purpose of developing a method that does not require naturally dry or air dried soil to undergo conditioning prior to a SIR assay.

Key Words
Soil, SIR, rewetting, amendment, conditioning.

Introduction

One method widely employed for monitoring soil microbial status is to quantify respiration in soil after the addition of a simple carbon source: Substrate Induced Respiration (SIR) (Anderson and Domsch 1987; Degens and Harris 1997). Miniaturisation of the assay by Campbell *et al.* (2003) allowed complex analyses of soil microbial "physiological profiles" at the community level (CLPP) to be performed quickly and inexpensively (for review see Chapman *et al.* 2007). CLPP has been used to assess the effects of pollutants (Kaufmann 2006), amendments (e.g. Degens *et al.* 2000) and landuse change (Lalor *et al.* 2007) on soils. However, while this method can contribute useful data to many areas of environmental science, agriculture and forestry; variability within and between soils becomes an obstacle to establishing a robust and versatile protocol. Soil conditioning has been used as one means of reducing this variability. It can also prevent the dramatic but short-lived increases in soil respiration occurring upon rewetting of dry soil (the "CO_2 burst") (Fierer and Schimel 2003) that impact the SIR assay. In our investigations of soil pretreatment and optimal CLPP assay conditions, we have observed that substrate addition during assays sometimes decreased, rather than increased, soil respiration. In order to explain these surprising results we examined the effects of various soil amendments, including some of the substrates used in SIR assays, on basal and substrate-induced respiration in sandy soil from the south-west of Western Australia (WA). We hypothesised that rewetting of the soil as well as addition of soil amendments will modify respiration levels and patterns in SIR assay.

Methods

Experimental setup and laboratory analyses

A sample of sandy forest soil (moisture content less than 5% and maximal water holding capacity approx. 0.6 g/ g dry soil) was collected from the Mt. Barker area (south-west of WA) and stored at between 15 and 25^{0}C for 8 weeks. To test the influence of soil amendments on soil respiration, 200 g portions of soil sieved through a < 2 mm mesh were mixed with different amendments and 50 ml water (where appropriate), placed in plastic containers and incubated for 4 weeks in the dark at 25^{0}C. The amendments used were simple carbon sources (D-glucose and organic acids: succinic, D-galacturonic and salicylic), supplied at 24 mg C/g soil; NPK fertiliser at 2.5 g/kg soil; herbicide (Muron 600) at 220 mg/kg soil; activated charcoal at 1:10 w/w and pine shavings at 1:3 v/v. Subsamples of soil for SIR assays were taken on the day of setup two hours after amendment addition and then on the seventh day of incubation (and after four weeks, data not shown). SIR assays were carried out in microplate format drawing from MicroResp^{TM} approach of Campbell *et. al* (2003). Six substrates were used at 18 mg C/g soil: thiamine, glucose, α-ketoglutaric acid, D-glacaturonic acid, imidazole and succinic acid. Due to low solubility in water, a saturated solution of cinnamic acid (the seventh substrate) was used. Control wells were amended with water only, which provided basal respiration measurement. The detection plate was prepared as recommended by Lalor (2007) except for some minor modifications. The results were expressed as µg CO_2-C/g oven dry soil/hr.

1 – 6 August 2010, Brisbane, Australia. Published on DVD.

Statistical analyses
T-tests and one-way ANOVA with Dunnett's C test for multiple comparisons (Quinn and Keough 2002) were used to compare differences between treatments, which were considered significant when $p<0.05$. Results of two separate experiments are presented.

Results
A "CO_2 burst" (five-fold increase in respiration as compared to dry soil) was observed two hours after re-wetting of unamended soil, but not after seven days. Of the amendments tested, only succinic acid did not inhibit this "CO_2 burst" (Figure 1a, left panel). During SIR assay two hours after rewetting, three of the seven assay substrates reduced soil respiration in control soil (rewetted without amendments), but their inhibitive effect was not observed after incubation for seven days. D-galacturonic acid inhibited soil respiration in the short term both as an amendment and as an assay substrate in rewetted soil (Figure 1a). For complex soil amendments, fertiliser, herbicide and charcoal inhibited respiration both in the short and long-term with levels closer to those of dry soil. However, pine shavings inhibited then stimulated respiration (in long-term) in a way similar to simple carbon sources (Fig 1 b, left panel).

The addition of the amendments not only altered the soil's response to re-wetting, but also its SIR profiles (Figure 1, right panel). Initially, the assay substrates increased respiration in soil amended with NPK and activated charcoal, and to a smaller extent with pine shavings, but not with glucose, succinic acid and herbicide. By the seventh day of incubation, only soils amended with pure water and pine shavings were responsive to only one of the assay substrates (succinic acid and glucose, respectively).

Discussion
Dramatic but short-lived increases in soil respiration after re-wetting, as shown in this study, have been well documented (Fierer and Schimel 2003). It was of concern that sudden rewetting of soil in a CLPP assay could elicit such a response and thus confound the results, "masking" the response to the substrate. However, low respiration level of dry soil (Figure1a, left panel) suggests that small amounts of water used in miniaturised SIR assays fail to elicit "CO_2 burst" and therefore are not likely to interfere with the assay.

Amending soil with simple (SIR substrates) and complex (charcoal and pine shavings) carbon sources prior to SIR assays impaired the soil's ability to respond to rewetting with a "CO_2 burst". These results were inconsistent with the hypothesis of "CO_2 burst" generation posed by Fierer and Schimel (2003) and therefore unexpected. Even more intriguing was the fact that some of the amendments (especially charcoal), despite inhibiting soil's response to rewetting, has altered soil's SIR pattern (Figure 1a, right panel). This suggested the activation of distinct microbial populations in soil. These preliminary results suggest the possibility of using the effect of diminishing of the "CO_2 burst" by substrates, added to soil upon re-wetting, for the purpose of detection of microbial community composition in dry soils (without conditioning or air dried).

Degens (1998) hypothesized that repeated addition of substrates as amendments to soil selects microbial community predisposed to utilisation of these substrates. His observation of additional increases in respiration caused by these substrates used in SIR assay is corroborated by our results (Figure 1b). It is interesting to note that soil amended with pine shavings, despite marked increase in basal respiration by the 7^{th} day of incubation, did not respond to most of the simple carbon sources in respiration assay (until four weeks of incubation, data not shown). It is likely that the complexity of the pine shaving substrate precludes the development of microbial community utilising simple carbon sources until they are released as the pine shavings degrade. Our results suggest that soil rewetting and subsequent incubation with or without amendments stimulate the development of different microbial communities. This is important if we consider that soil conditioning (re-wetting and incubation of soil before microbial analyses) aims at reducing variation in soil microbial analyses. Our results suggest that such a pretreatment may selectively encourage growth of microbial populations (depending of the quality and quantity of microbial substrates available in the soil, as suggested by Degens 1998), therefore becoming a source of additional variation, and thus have important implications for the interpretation of the results of CLPP analyses.

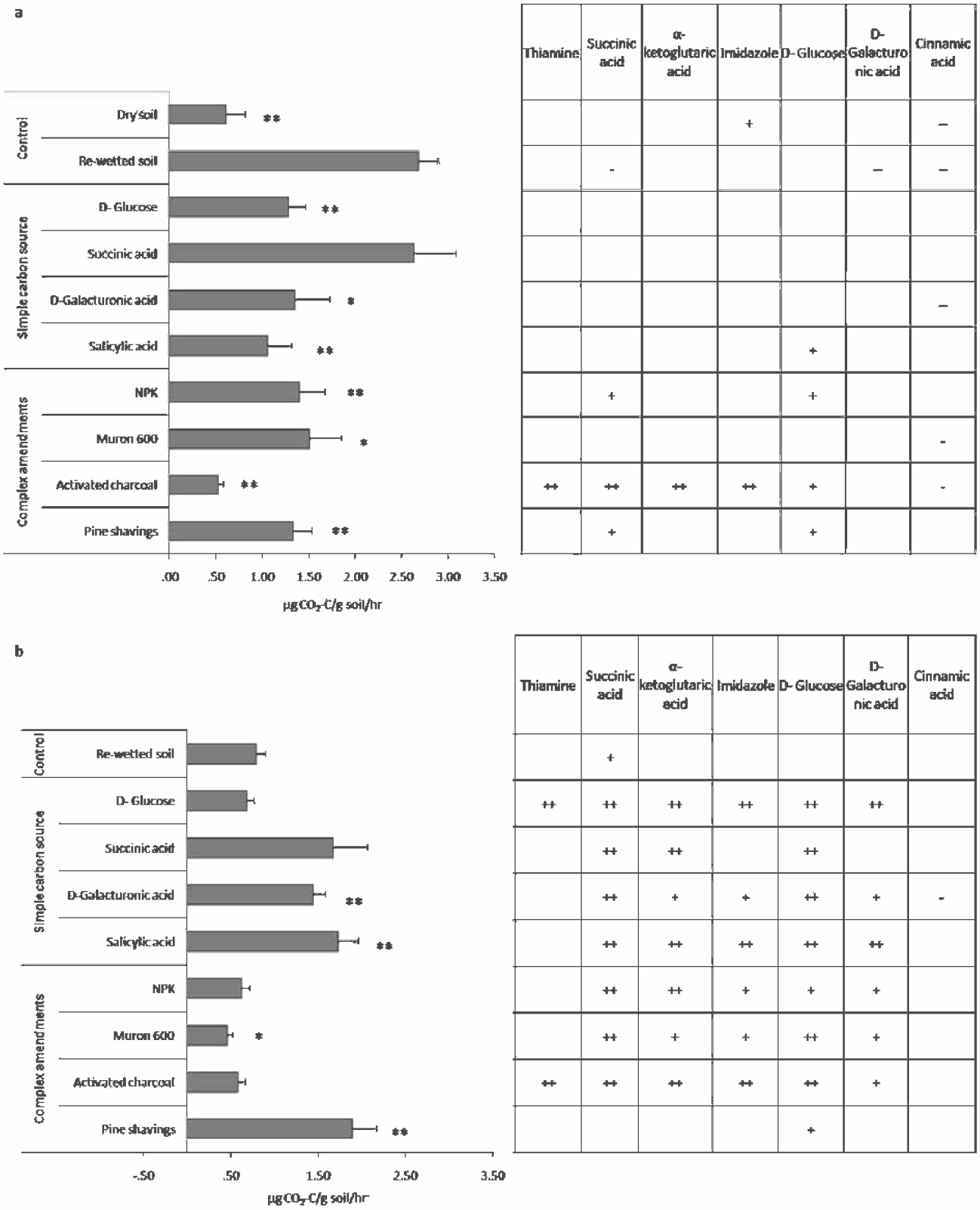

Figure 1. Basal (left panel) and substrate-induced respiration (right panel) of soil after addition of amendments; (a) 2 hrs and b) 7 days of incubation at 25^{0}C. Error bars denote standard error of the mean. Left panel - difference from control (soil re-wetted with water); * - p<0.05; ** - p<0.01. Right panel - the increase (+) and decrease (-) in respiration as compared to control (water without substrate) in substrate-induced respiration assay are represented by single (p<0.05) or double (p<0.01) symbols.

Conclusion

This study demonstrated that the "CO_2 burst" observed upon rewetting of dry soil can be alleviated by amending soil with a variety of substances; however, the mechanisms behind this are not clear. Taking into account that conditioning can significantly alter soil microbial communities, further investigation of "CO_2

burst inhibition" is warranted for the purpose of developing a method that is suitable for SIR analysis of naturally dry or air-dried soils: one that would not require soil conditioning.

Acknowledgements
This work was supported by Murdoch University Research Scholarship with top-up from the CRC for Forestry. We thank the Western Australian State Agricultural Biotechnology Centre at Murdoch University for access to the equipment.

References

Anderson JPE, Domsch KH (1978) Physiological method for quantitative measurement of microbial biomass in soils. *Soil Biology and Biochemistry* **10**, 215-221.

Campbell CD, Chapman SJ, Cameron CM (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. *Applied and Environmental Microbiology* **69**, 3593-3599.

Chapman SJ, Campbell CD, Artz RRE (2007) Assessing CLPPs using MicroReSp (TM) - A comparison with biolog and multi-SIR. *Journal of Soils and Sediments* **7**, 4

Degens BP, Harris JA (1997) Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. *Soil Biology and Biochemistry* **29**, 1309-1320.

Degens BP (1998) Microbial functional diversity can be influenced by the addition of simple organic substrates to soil. *Soil Biology and Biochemistry* **30**, 1981

Degens BP, Schipper LA, Claydon JJ (2000) Irrigation of an allophanic soil with dairy factory effluent for 22 years: responses of nutrient storage and soil biota. *Aust. J. Soil Res.* **38**, 25-35.

Fierer N, Schimel JP (2003) A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. *Soil Sci. Soc. Am. J.* **67**, 798–805.

Kaufmann K, Chapman SJ, Campbell CD, Harms H, Hohener P (2006) Miniaturized test system for soil respiration induced by volatile pollutants. *Environmental Pollution* **140**, 269-278.

Lalor BM, Cookson WR, Murphy DV (2007) Comparison of two methods that assess soil community level physiological profiles in a forest ecosystem. *Soil Biology and Biochemistry* **39**, 454-462.

Quinn GP, Keough MJ (2002) 'Experimental design and data analysis for biologists' (Cambridge University Press: Cambridge, UK, New York).