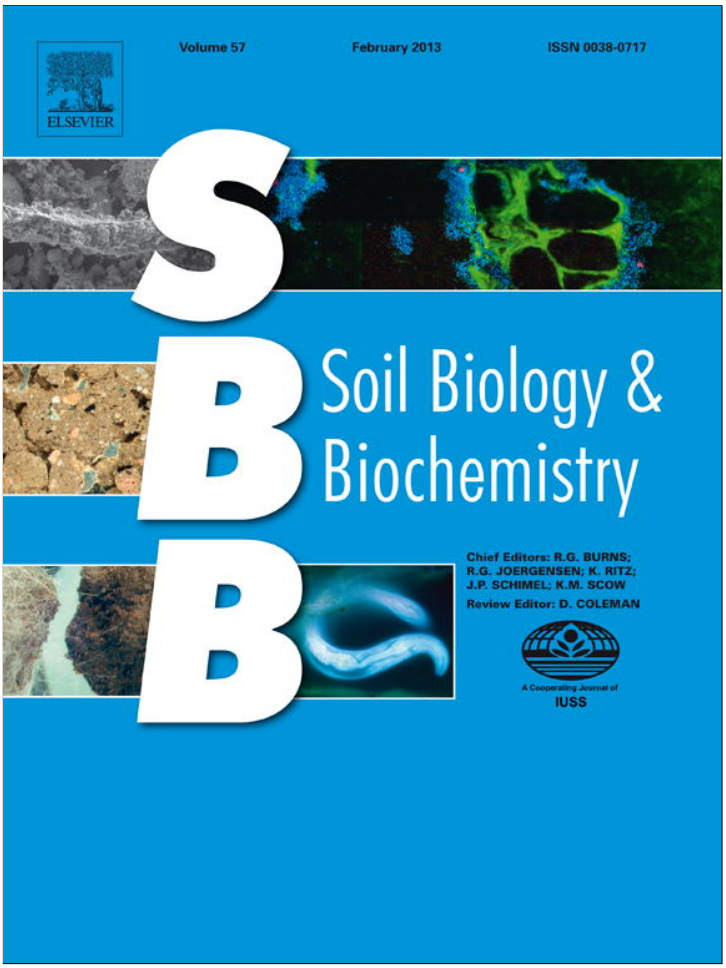

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Short communication

Application of a high-throughput laboratory method to assess litter decomposition rates in multiple-species experiments

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ABSTRACT

Laboratory decomposition experiments measuring the mineralization of C from plant residue are increasingly used to test the effects of different litter sources and soil conditions. To date, the most widely used methods are infra-red gas analysis (IRGA) and gas chromatography, which are time-consuming, and relatively expensive and complex to operate. The aim of this study was to devise a simple and rapid laboratory method to assess litter decomposition rates at frequent time intervals. We modified the MicroResp™ system to measure the amount of CO_2 produced by two soils supporting contrasting microbial communities and containing the litter of eight crop species. The method was sensitive enough to differentiate both soil microbial communities and litter qualities. The method combines the accuracy of gas chromatography with the speed of absorbance measurements obtained via microplate readers. This technique provides an effective means for devising complex litter decomposition experiments capable of addressing the joint influence of multiple species, soil communities, environmental conditions, and their multiple interactions.

In terrestrial ecosystems, more than 50% of net primary production is transferred to the soil via the litter decomposition pathway (Wardle et al., 2004). The pace of this transfer is known to be controlled by a suite of factors acting at different scales, chiefly climate, litter chemistry and soil biota (Hättenschwiler et al., 2005; Cornwell et al., 2008). Research on litter decomposition has provided key decomposition metrics that are readily comparable among litter sources and soil conditions, at least within individual experiments. This has led to the rise of decomposition studies run under controlled laboratory conditions. In these microcosm studies, litter decomposition is considered as the mineralization of C from plant residue, measured as CO_2 production (Aerts and de Caluwe, 1997). To date, the most widely used methods to measure CO_2 production in litter incubations are infra-red gas analysis (IRGA) and gas chromatography. These methods are more accurate than classical estimations of soil respiration using soda lime (Teuben, 1991) or alkali traps (Froment, 1972), but are time-consuming, relatively expensive and complex to operate, requiring specific skills (Mondini et al., 2010). Also, the fact that they are time-consuming precludes their widespread use in large multi-species screening experiments. The aim of this study was to devise a simple and rapid laboratory method to assess litter decomposition rates at frequent time intervals. We assessed the reliability of the method by testing a gradient of litter quality and two soils with contrasting microbial communities, where an increase in decomposition was anticipated via higher litter quality and higher soil microbial functional diversity.

We analyzed soil respiration, a measure of decomposition, with a modified procedure of the MicroResp™ system (Campbell et al., 2003). This is a whole-soil method based on community level physiological profiles obtained by testing different carbon sources of contrasting recalcitrance. The substrate utilization rates of the carbon sources correspond to the catabolic activity of the microbial community and have been used to analyze microbial functional diversity (Ginzburg et al., 2008) or heterotrophic evenness (Schipper et al., 2001). In this study, we modified the MicroResp system to measure the amount of CO_2 that is being respired by soil

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microorganisms decomposing plant litter. In summary, the system is based on the use of a CO_2 detection solution containing cresol red indicator dye. The whole method relies on the color change experienced by the indicator solution as a consequence of the changes in pH occurring when respired CO_2 reacts with the bicarbonate. Therefore, although counterintuitive, the absorbance of the detection solution will decrease as cresol red changes from pink to yellow with higher CO_2 concentration. We set aliquots of 150 μL of purified agar (2.5%), cresol red indicator dye (18.8 ppm), potassium chloride (225 mM) and sodium bicarbonate (3.7 mM) in each well of a microplate strip (1 × 8 breakable).

Naturally senesced leaf litter was collected from eight crop species with contrasted litter qualities (Table S1). Two roadside grasslands from central Spain differing in the road construction age were selected to represent different successional stages, and hence soil with contrasted microbial communities (Table 1). Aliquots (0.75 g) of dried litter and 60 g of fresh soil and were introduced into 250 mL air-tight Mason jars, where moisture was adjusted to 50% water holding capacity (see Supplementary material for details on microcosm construction). Microcosms were placed in trays and introduced in a growth chamber for incubation in dark conditions at 20 °C and 100% humidity (Fig. S1A). Two 'no-litter' microcosms per soil type were placed in each tray to correct for soil contribution to CO_2 production, totaling 10 'no-litter' microcosms per soil type. For this aim, CO_2 production in the 'no-litter' microcosms in each tray was subtracted from all the 'litter + soil' microcosms in that tray (Strickland et al., 2009).

To calibrate the color change experienced by the indicator solution, 36 soil samples were measured in parallel with the colorimetric method (modified procedure of the MicroResp system) and a gas chromatograph (Varian CP-3380). The gas chromatograph was equipped with a sampling valve (250 μL, with heating oven) and two columns (Hayesep Q and Molecular Sieve 13X), using He as the carrier gas. A very strong relationship was found between the peak area and the concentration of the standard CO_2 (Fig. 1A), qualifying use of the peak area of the samples in the colorimetric-gas chromatograph calibration. To promote different soil respiration rates, and hence contrasted % CO_2, different amounts of the two soils were

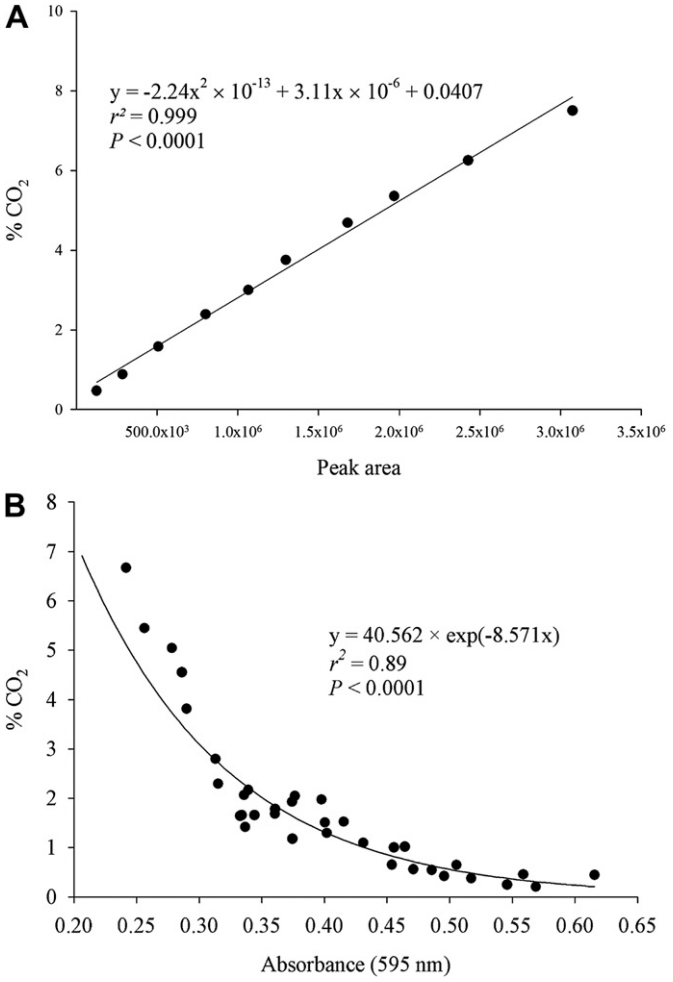

Fig. 1. Calibration of the color change experienced by the indicator solution with the gas chromatograph. A) Relationship between the peak area and the concentration of the standard CO_2 (n = 10). B) Calibration curve for absorbance *vs.* percentage of CO_2 (n = 36).

Table 1
Site characteristics and soil description of the two roadside grasslands. Data for soil variables are means ± 1 SE (n = 10).

	Early (0–2 years)	Late (>20 years)
Coordinates (U.T.M.)	30S 0482078/4405352 N	30T 0424133/4469923 N
Elevation (m a.s.l.)	731	615
pH	8.31 ± 0.29	7.15 ± 0.17
Total N (mg N g soil^{-1})	0.78 ± 0.07	2.44 ± 0.16
Total P (mg P g soil^{-1})	0.26 ± 0.01	0.65 ± 0.04
Organic C (mg C g soil^{-1})	7.66 ± 0.67	23.02 ± 1.28
Microbial functional diversity[a]	1.881 ± 0.51	2.627 ± 0.84
Bacteria (DNA copies g^{-1} soil)[b]	1.36 10^8 ± 8.52 10^7	3.56 10^9 ± 1.31 10^9
Fungi (DNA copies g^{-1} soil)[b]	9.24 10^6 ± 3.71 10^6	9.27 10^8 ± 4.40 10^8
Relative fungal: bacterial ratio	0.07 ± 0.01	0.26 ± 0.02

[a] The functional diversity of the soil microbial communities was quantified using a carbon substrate diversity index or modified Shannon index from the data gathered in García-Palacios et al. (2011) with the MicroResp system: $H' = -\Sigma[pi \ln(pi)]$, where: pi is the ratio of the CO_2 rate for a carbon source to the sum of CO_2 rates for all substrates.

[b] The relative abundance of bacterial 16S and fungal 18S rRNA genes was measured using quantitative PCR (García-Palacios, unpublished data). The bacterial and fungi genes were amplified with the Eub 338–Eub 518 and ITS 1–5.8S primer sets, respectively following Fierer et al. (2005).

introduced in 100 mL Duran® bottles sealed with a rubber bung, adjusted to 50% WHC, and mixed with different volumes of three carbon sources (D-fructose, L-alanine and citric acid). One 4-well microstrip filled with the indicator solution was attached to each bottle side. After a 6 h period, CO_2 produced was extracted with a syringe and measured on the gas chromatograph. Absorbance of microstrips was read at 595 nm immediately before and after the 6 h period. Although this wavelength has shown good performance in previous applications of the MicroResp system (García-Palacios et al., 2011), the optimal wavelength for this indicator dye is 572 nm, as described in the MicroResp Technical Manual (Macaulay Institute, Aberdeen, UK). The calibration of dye color with % CO_2 gave a highly significant negative exponential relationship (Fig. 1B) that obviated the need to use gas chromatography to determine respiration rates subsequently during the course of the experiment. Greater concentrations of the detection solution reactants should be used whether soil respiration rates are high enough to promote drastic decreases in absorbance (yellow color), because the exponential calibration curve fits poorly in these situations (Fig. 1B). Based on this calibration, microcosm CO_2 concentrations above 3% would indicate the need to increase the concentration of the detection solution, which was not the case in our experiment.

Soil respiration was measured over a 66-day period, daily during the first week and weekly over the course of the experiment. At

each sampling date, jars containing the 4-well microstrips (Fig. S1B) were closed air tight for 6 h and absorbance was read at 595 nm immediately before and after that period. The well absorbance after 6 h was normalized for any differences recorded at zero time before exposure, averaged in each jar, and then converted to the headspace CO_2 concentration by using the calibration curve. CO_2 concentration (%) was converted to CO_2 production rate (μg CO_2–C g soil^{-1} g litter^{-1} h^{-1}) by using the gas constant, incubation temperature, headspace volume in the microcosms, fresh weight of soil, dry litter weight, incubation time and soil sample % dry weight (Campbell et al., 2003). Newton integration was applied to the CO_2 production rate to calculate the cumulative respiration (mg CO_2–C g soil^{-1} g litter^{-1}). This integration is useful when the soil respiration has been measured at different time scales (daily *vs.* weekly), and after the average CO_2 production rate for each time interval between two measuring dates has been computed (Milla et al., 2006). We analyzed the cumulative respiration at the end of the experiment using a two-way ANOVA, with plant species and soil as fixed factors. Pearson correlations were used to study the relation between cumulative respiration and litter quality parameters. Relationship between % CO_2 and absorbance was evaluated using an exponential regression model. Analyses were carried out using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA).

Soil and plant species significantly affected the cumulative respiration ($F_{1,64} = 39.87$; $P < 0.0001$ and $F_{7,64} = 7.61$; $P < 0.0001$, respectively). However, the interaction between them was not significant ($F_{7,64} = 1.44$; $P = 0.205$). Cumulative respiration was higher in the late-successional soil microbial communities, irrespective of the source litter (Fig. 2). This result has been previously found in the literature (Strickland et al., 2009; Sherman and Steinberger, 2012), but most importantly, highlights the sensitivity of the method tested to detect the impacts of microbial community composition on decomposition. Post-hoc tests revealed a greater cumulative respiration in *Zea*, *Lycopersicum*, *Lactuca* and *Amaranthus*. When the relationship between cumulative respiration and litter C:N was examined separately for each soil, a significant correlation was found in the early-successional soil ($\rho = -0.715$; $P = 0.046$; $n = 8$), but not in the late-successional one ($\rho = -0.238$; $P = 0.570$; $n = 8$). Therefore, the ability of litter quality to predict litter decomposition was determined by the microbial community in the soil (Strickland et al., 2009). Altogether, the above results suggest that this method is sensitive and precise enough to differentiate soil respiration rates in microcosms with contrasted soil microbial communities and litter qualities.

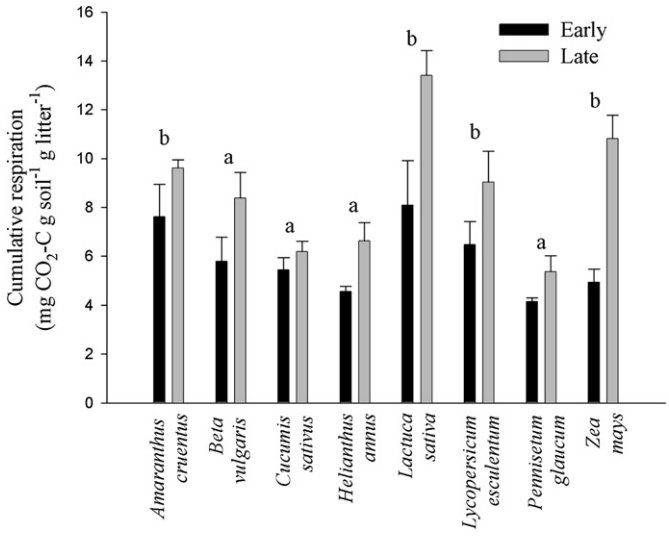

Fig. 2. Cumulative respiration from microcosms consisting of eight species litter combined with either the early- or the late-successional soil. Values are means ± SE ($n = 5$). Data were log-transformed to reach normality. Lowercase letters indicate significant differences among species at $P < 0.05$ (Tukey's HSD test).

A critical issue for the applicability of the method is its ability to differentiate the CO_2 production derived from the degradation of litter from the CO_2 coming from the basal soil respiration. We found that far greater amounts of CO_2 were respired from microcosms that contained leaf litter and soil than from the control 'no-litter' microcosms (Fig. S2), indicating that most CO_2 was derived from decomposing litter ($\approx$70% in both soils). This method is thus able to detect the effects of small amounts of litter inputs entering the microbial food web (0.75 g of litter added to 60 g of soil). However, the particular conditions of the soils used to test this method (weakly oxygenated and alkaline soils) could involve the risk of ammonia production and/or abiotic CO_2 release interfering with the color change of the detection solution. These potential artifacts should be taken into account when applying this method in a wider ecological context. Alternative methods able to measure soil respiration at frequent time intervals, such as IRGA or gas chromatograph, are time-consuming since they need certain accumulation time to calculate the CO_2 flux in each sample. However, we are not comparing different methods to measure accumulated CO_2 during litter decomposition. Instead of that, we have devised a high-throughput laboratory method that combines the accuracy of gas chromatography, with the speed of absorbance measurements attainable with a microplate reader. Critically, the use of 96-well plates allows the measurement of large experiments in one day ($n = 300$; García-Palacios, unpublished data). In conclusion, this simple and rapid laboratory method opens new avenues for litter decomposition research because it allows the realization of complex experimental designs, e.g. with multiple factors otherwise difficult to manage with existing methods.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2012.09.029.

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