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Organic textile dye improves the visual assessment of the bait-lamina test

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ABSTRACT

Rapid ecosystem assessments are needed for large-scale ecotoxicological studies and coordinated distributed experiments. Bait-lamina stripes are commonly used as a standardized method to assess decomposer activity, but it is often difficult to distinguish bait substrate from soil. In the present study our aim was to identify a dyeing method that improves the precision of visual assessment of decomposition rates, while having negligible side effects. We compared five different dyes (food dye, Easter Grass, organic textile dye, ink, and wall paint) with control substrate in microcosms containing either acidic or alkaline soil with two introduced Collembola species (*Folsomia candida* and *Sinella coeca*). Organic textile dye showed the highest precision of visual assessment, and had no detectable side effects on decomposition rates, soil microbial activity (biomass and respiration), or Collembola densities. We recommend using organic textile dye to improve the bait-lamina test due to the high precision and the ease of preparation.

1. Introduction

To assess the global nature of many environmental and ecotoxicological problems, experiments are increasingly being replicated in multiple locations around the world (Kools et al., 2009; Fraser et al., 2013). Such globally coordinated distributed experiments require standardized methods that can be done rapidly and inexpensively in the field. The bait-lamina test (Von Törne, 1990) is one such method that is often used to reflect the feeding activity of detritivorous micro- and mesofauna in the soil (Gestel et al., 2003; Hamel et al., 2007; Birkhofer et al., 2011). Improving the bait-lamina test method would facilitate rapid assessment of decomposition of organic materials, which is arguably one of the most important ecosystem processes on Earth.

Despite low costs and easy handling of bait-lamina stripes, field assessments of decomposition rates can be complicated because the bait material is often difficult to distinguish from soil. Thus, the objective of the present study was to develop a cost-efficient and manageable dyeing method of bait-lamina substrate that would not influence soil organisms, side effects that could bias results of field tests. To assure that the new dyeing method could be broadly applied across a range of conditions, we tested the methods in two soil types differing in pH.

2. Material and methods

2.1. Bait-lamina stripes and dyeing methods

Bait-lamina stripes (Terra Protecta GmbH) consist of 16 cm long PVC stripes with 16 holes of 2 mm in diameter (Kratz, 1998). Holes were filled repeatedly with the respective substrate to ensure constant volume (Kratz, 1998). The substrate (Terra Protecta GmbH) consisted of 70% cellulose (micro-granular), 27% bran flakes (<500 μm), and 3% activated carbon. We used 2 g substrate and mixed it with different dyes: ink (4 ml; red; Pelikan 4001 Brillant-Rot; Pelikan GmbH; Germany), organic textile dye (3 ml;

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white; based on natural components; Livos Pflanzenchemie GmbH & Co. KG, Germany), wall paint (0.9 g; white; consisted of lime-casein; Kreidezeit Naturfarben GmbH; Germany), food dye (5.5 ml; yellow; Wusitta für Lebensmittel; Germany), and ground Easter Grass (1 g; green; Brauns-Heitmann GmbH & Co. KG, Germany). After mixing each dye with the control substrate, we added a specific amount of water to get comparable consistency of the bait mass. Dyed baits and control baits were dried at 40 °C for one hour.

2.2. Experimental design and measurements

We tested the effects of six bait-lamina substrates (control substrate, Easter Grass, food dye, ink, organic textile dye, wall paint), and two soils (alkaline and acidic) on readability and on side effects on the density and activity of soil organisms using a completely randomized design microcosm experiment. Each of the six treatments (six bait substrates) was replicated five times in two soil types, ending up with 60 microcosms in total. Microcosms consisted of PVC tubes (diameter: 10 cm; height: 25 cm; 0.45 mm gaze at the bottom) filled to a height of 20 cm with soil (~1.5 kg of fresh soil). Microcosms were surrounded by a transparent plastic enclosure (15 cm height), stuck to the upper rim of the microcosm with adhesive tape to prevent added Collembola from escaping. We inserted three bait-lamina stripes per microcosm (one type of bait substrate) in a triangular pattern with a distance of about 2 cm from the inner edge of the microcosm. Three stripes were used to cover some spatial heterogeneity in the microcosms, but only the mean per microcosm entered the statistical analyses to avoid pseudo-replication. To ensure an equal vertical insertion depth of 10 cm, we marked the stripes at 1 cm above the last hole (note slight modification to Von Törne, 1990).

To assess the precision of the test in different environmental conditions we established two different soil treatments. Microcosms were filled with either acidic soil derived from Kreinitz, Germany (pH ~5.5, nutrient-poor Cambisol; 11°5′E, 50°54′N) or alkaline soil from Jena, Germany (pH ~8.1, Eutric Fluvisol; 50°57′N, 11°35′E). All soils were hand sorted to remove larger stones and roots, kept moist throughout the experiment by adding the same amount of water to each microcosm when the soil surface appeared to be dry, and maintained at 20 ± 2 °C in a climate chamber (day/night: 16/8 h; greenhouse lamps, 1000 W, 50 Hz).

To assess potential side effects of dye types on soil microorganisms and mesofauna, in addition to micro- and mesofauna being already present in the soil, we introduced two Collembola species *Folsomia candida* Willem and *Sinella coeca* Schött from laboratory cultures. Those two species were added to assess feeding activity of Collembola because they represent different ecological strategies: the epedaphic species *F. candida* (Isotomidae) lives and feeds on the soil surface, while the hemiedaphic species *S. coeca* (Entomobryidae) lives and feeds in the soil and on the soil surface. Twenty individuals of each species and of similar age were added to all microcosms one week before the bait-lamina stripes were inserted to ensure high decomposer activity and to have a standard measure of potential side effects on soil microarthropods.

Microcosms were randomly distributed after the Collembola species had been introduced and again after the first assessment of decomposer activity (one week after the addition of Collembola). After the second assessment of decomposer activity (two weeks after the addition of Collembola), we took three soil cores to a depth of 5 cm from each microcosm to test for potential side effects on soil microorganisms and mesofauna. One core of 5 cm diameter was used for heat extraction of Collembola (Kempson et al., 1963). Extracted individuals per core were stored in 70% ethanol and counted. The soil of the two other cores (2 cm diameter) was pooled and sieved, to measure basal respiration (BR; $\mu l\ O_2\ h^{-1}\ g^{-1}$ soil dry weight), microbial biomass carbon (C_{mic}; $\mu g\ C_{mic}\ g^{-1}$ soil dry weight), and specific respiration (qO_2; $\mu l\ O_2\ mg^{-1}\ C_{mic}\ h^{-1}$) in an O_2-microcompensation apparatus (Scheu, 1992) as described in Eisenhauer et al. (2009).

To assess decomposer activity, bait-lamina stripes were removed from soil one and two weeks after the experiment had started (see above). According to the manufacturer's recommendation, we recorded decomposition rates of the substrate in each hole and rated it as "0" when the substrate was not perforated, as "0.5" when the substrate was perforated partially, and as "1" when the substrate was removed completely. After the first measurement, we put the stripes back into the respective microcosms. The precision of visual assessment of the dyeing methods was evaluated by ten university students using three randomly chosen stripes of each dyeing method (stripes did not vary systematically in the number of empty holes). A measure of confidence was assessed using a scale from one (not distinguishable) to five (easy to distinguish) to assess whether bait-lamina substrate was distinguishable from soil.

2.3. Statistical analyses

We used sequential analysis of variance (ANOVA, type I sum of squares) to test the effects of five dyeing methods and control substrate on the measure of confidence. The sequential approach tested effects of dyeing method after accounting for the variance explained by student ID to preclude potential confounding effects of student ID. Tests with and without student ID yielded very similar results indicating the robustness of our findings (not shown). Further, repeated-measures ANOVA was used to test the effects of time (week 1 and week 2), soil type, dyeing method, and the interaction between soil type and dying method on decomposer activity (number of empty holes per stripe). To assess potential side effects of the dyeing methods on soil microorganisms and mesofauna, we used ANOVA to test the effects of soil type, dyeing method and the respective interaction on soil microbial basal respiration, biomass C, specific respiration and Collembola density

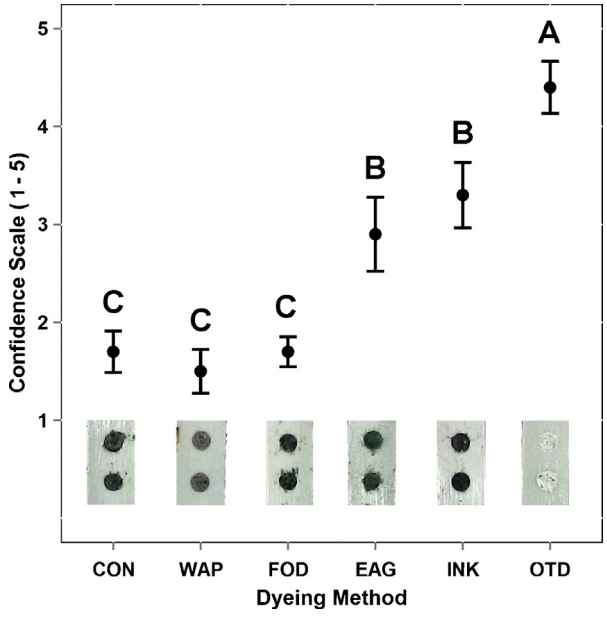

Fig. 1. Measure of confidence of visual assessment of decomposer activity using bait-lamina stripes as affected by dyeing method (WAP = wall paint, FOD = food dye, EAG = Easter Grass, INK = ink, OTD = textile dye). The precision of visual assessment was evaluated by ten university students using a scale from one (not distinguishable) to five (easy to distinguish) to assess whether bait-lamina substrate was distinguishable from soil. Means with standard errors. Bars with different letters vary significantly (Tukey's HSD test, $p < 0.05$).