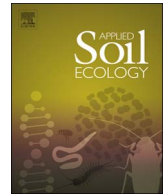




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Inoculation of an ecosystem engineer (Earthworm: *Lumbricus terrestris*) during experimental grassland restoration: Consequences for above and belowground soil compartments

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ABSTRACT

Although soil organisms might strongly affect the dynamics and composition of natural vegetation, relatively few studies have tried to in-situ manipulate soil fauna, especially in restoration ecology. The objective of this study was thus to observe the impact of a soil ecosystem engineer (*Lumbricus terrestris* L.) on plant communities as well as on soil organisms (springtails) in a reclaimed floodplain previously devoid of earthworms. Within a randomized factorial design based on buried frames (depth 0.45 m), half of the quadrats (1 m²) were inoculated with 100 earthworms, the other half served as control. After one year of experiment, earthworm inoculation doubled the plant biomass and favored grass species over forbs. Both abundance and diversity of Collembola (depending on functional groups) were negatively impacted by the presence of earthworms. Using a path analysis we found that this negative impact was probably indirect and due to an earthworm effect on plant community structure and plant functional groups. We suggest in our particular case that vegetation, and more precisely plant biomass and functional traits, may be more influential than soil properties in driving Collembola assemblages. Regarding restoration, we conclude that manipulating earthworms could be an interesting tool for increasing plant productivity but may disfavor soil biodiversity and alter above-belowground linkages.

1. Introduction

Over the last decade, an increasing number of conceptual and empirical studies have stressed the importance of soil fauna and above-belowground linkages in driving communities and ecosystem properties (Bardgett and Wardle, 2003; Wardle et al., 2004). For example, invertebrate soil fauna might enhance both secondary succession and local plant species diversity (De Deyn et al., 2003). The composition and diversity of soil fauna communities also determine ecosystem multifunctionality (Wagg et al., 2014). As a consequence, it has been suggested to consider soil biota and above-belowground linkages to assist conservation and restoration ecology (Kardol and Wardle, 2010).

To date, the manipulation of soil fauna in restoration ecology has concerned earthworms as emblematic soil ecosystem engineers (sensu Jones et al., 1994). Through their feeding and burrowing activities, they influence soil physical properties such as aggregate stability, soil structure, infiltration of water, and aeration of deeper soil layers.

Earthworms also modify soil biotic properties such as microbial biomass and activity, nutrient cycling and mineralization, density of other soil invertebrates, plant productivity and community composition, and aboveground food webs (for a complete review see Blouin et al., 2013). The inoculation of earthworms in soils devoid or with a low density of earthworms may be a tool for assisting the rehabilitation of degraded lands (Butt, 1999; Snyder and Hendrix, 2008; Boyer and Wratten, 2010; Jouquet et al., 2014). However, a large majority of these studies focused on earthworm inoculation techniques (choice of earthworm species, density, methods, timing, costs, etc.; for a review see Butt, 2008), and only looked at the restoration of soil properties such as aggregate structure and soil porosity (Fraser et al., 2003; Marashi and Scullion, 2003), soil fertility (Scullion and Malik, 2000; Fraser et al., 2003) or remediation of contaminated soils (Sizmur et al., 2011). In comparison, studies that used earthworm inoculation to assist restoration of the biotic (above and/or belowground) component of an ecosystem are rather scarce (e.g. Curry and Boyle, 1987; Roubickova et al.,

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2009; Mudrak et al., 2012). And only one of them tested the effect of earthworms on both late successional plants and soil fauna (Mudrak et al., 2012). In this 3-month laboratory pot experiment, these authors demonstrated that the inoculation of earthworms could contribute to drive the succession of plant and soil fauna communities (Collembola).

Among belowground organisms Collembola (springtails) constitute a model soil organism group (e.g. Henneron et al., 2017). They are the most abundant soil invertebrates present in almost all terrestrial ecosystems. By interacting with microorganisms through fungal grazing and others mechanisms, these soil microarthropods are recognized to play an important role in litter decomposition, nutrient cycling and plant growing processes (Petersen, 2002; Partsch et al., 2006; Forey et al., 2015). Collembolan species are generally classified into three life-form groups (epedaphic, hemiedaphic and euedaphic) according to their ecology and sensitivity to environmental conditions (Gisin, 1943; Petersen, 2002), which allow to investigate their functional assemblages. Recent studies demonstrated that plant community structure and functions could be strong drivers of collembolan assemblages (Abgrall et al., 2017; Henneron et al., 2017). Indeed, according to the plant functional diversity hypothesis (Balvanera et al., 2006; Eisenhauer et al., 2010b), rich plant communities could favor decomposer diversity due to enhanced microhabitat and substrate heterogeneity (Wardle et al., 2005). Alternatively, the plant mass-ratio hypothesis (Grime, 1998) states that soil fauna assemblages should be driven primarily by traits of the dominant plant species (those contributing most to productivity; Wardle et al., 2005).

The objective of our study was to assess the importance and impact of inoculated earthworms (*Lumbricus terrestris* L.) on above and belowground compartments, i.e. on plants and on Collembola. This study was set within a larger restoration project that aimed at using former exploited gravel pits to restore wetland habitats or grasslands to be used as pastures for cattle grazing or mowing (Mchergui et al., 2014). In this reclaimed land, earthworm abundance was around zero. Thus, the inoculation of earthworm as an ecosystem engineer in this site could facilitate ecosystem dynamics to recreate an herbaceous ecosystem. After one year of experimentation, we monitored plant and Collembola communities in plots inoculated or not with *L. terrestris*. We hypothesized that earthworms might (1) indirectly enhance plant productivity through increasing soil fertility, (2) drive plant species assemblages by favouring some functional types (3) and thus directly or indirectly

modify Collembola communities with contrasting responses according to their functional group (Fig. 1).

2. Materials and methods

2.1. Study area and restoration project

The experiment was conducted in a currently exploited gravel quarry ('Carrières et Ballastières de Normandie') in Yville sur Seine (49° 29' 05' N; 00° 52' 31" E) located in the floodplain Seine valley in France. Average daily temperatures range from -5°C in winter to 27°C in summer. For decades, these gravels have been dug out from pits that are naturally filled with water after their use. Recently, a new project aimed at filling pits with a deep layer of sediments dredged out from the nearby Seine River, which is then covered by alkaline peat to restore local grasslands for grazing or mowing. Thereby, collective industrial objectives of dredging the Seine, exploiting gravels and ecological objectives of restoring grasslands can be met.

After been exploited, a gravel-pit was progressively filled between 2009 and the end of 2011 with sediments dredged from the Seine River (between 7 and 8 m) and covered with a layer of sand (10 cm, to stabilize sediments). Then, to reconstitute the initial soil profile, a layer of peat coming from adjacent wet meadows (between 70 and 80 cm) was added. This is an alkaline alluvial peat naturally deposited during the Holocene (Mchergui et al., 2014). Lastly, the peat was topped with a layer of grassland soil (10 cm). This topsoil corresponded to the a mixture of the organic layers of a soil called "hemic histosol covered by clay alluvial deposits" (Mchergui et al., 2014) that was present before the exploitation of a new gravel pit. This topsoil was collected in wetlands located less than 500 m away, which correspond to the desired grassland. The potential C and N mineralization of the peat was estimated in aerobic conditions and respectively ranged between 98 and $348\ \mu\text{g d}^{-1}\ \text{g}^{-1}$ and between 4 to $8.1\ \mu\text{g d}^{-1}\ \text{g}^{-1}$ of dry soil.

Vegetation was absent in this site during the first months of the experimentation. Earthworm density observed on another gravel-pit refilled with a comparable process (but without topsoil) was very low ($0.27 \pm 0.49\ \text{ind m}^{-2}$) after 3 years of monitoring (Grand Port Maritime de Rouen (GPMR), 2013) in comparison to nearby permanent wet grasslands ($1082 \pm 363\ \text{ind m}^{-2}$). This allowed us to state that earthworms were almost absent from our site just after the refilling

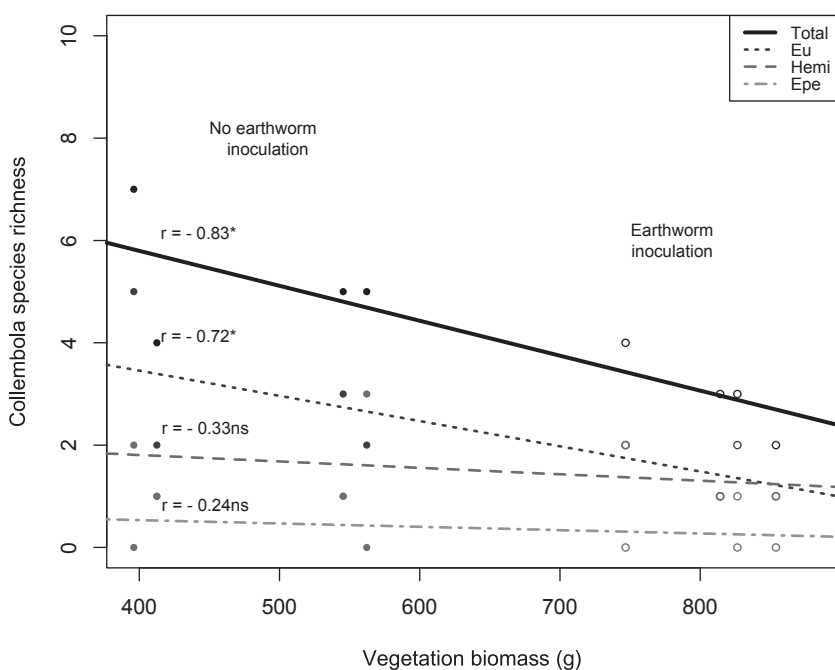


Fig. 1. Impact of plant biomass on total Collembola species richness (Total), and species richness of Euedaphic (Eu), Hemiedaphic (Hemi) and Epedaphic species (Epe) in plots inoculated with earthworms (white points) and plots not inoculated with earthworms (black points). Correlation coefficients (r) and level of significance ($\alpha = 0.05$) are indicated for each relation: * $p < 0.05$, ns: non-significant.

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