Can Lumbricus terrestris be released in forest soils degraded by compaction? Preliminary results from laboratory and field experiments

Vincent Ducasse, Frédéric Darboux, Appoline Auclerc, Arnaud Legout, Jacques Ranger, Yvan Capowiez

Can *Lumbricus terrestris* be released in forest soils degraded by compaction? Preliminary results from laboratory and field experiments

Vincent Ducasse [a,b], Frédéric Darboux [a,b,†], Appoline Auclerc [a],

Arnaud Legout [b], Jacques Ranger [b], Yvan Capowiez [c,*]

[a] Université de Lorraine, INRAE, LSE, F-54000 Nancy, France

[b] INRAE, BEF, F-54280 Champenoux, France

[c] INRAE, EMMAH, UMR 1114 INRAE-Avignon University, Domaine Saint Paul, F-84914 Avignon cedex 09, France

[†] Presently at: Univ. Grenoble Alpes, INRAE, ETNA, F-38402 St-Martin-d'Hères, France

[*] Corresponding author:

yvan.capowiez@inrae.fr

(+33) 4 72 34 24 38

Compaction is an important and increasing threat for forest soils. In addition to preventive approaches, remediation solutions are needed. Among them, the release of anecic earthworms is thought to be a promising approach. However, most previous attempts in compacted forest soils failed to retrieve the introduced earthworms. To gain more insight on the feasibility of releasing *Lumbricus terrestris* adults, we performed both a laboratory and a field study. Under laboratory conditions, the *L. terrestris* behavior was assessed in repacked soil cores with increasing soil bulk density (1.3, 1.4, 1.5 and 1.6 g cm^{-3} for a silt-loam soil). We found that burrowing (burrow volume, diameter and maximal burrow depth) as well as casting and feeding behavior were significantly reduced (-95%, -50% and -65%, respectively) at the highest bulk density compared to the lowest. For the field study, we released *L. terrestris* in a 10-year old experimental forest site in which two factors were studied: soil compaction and liming. To prevent them from escaping, the earthworms were caged in PVC cylinders in compacted (or not) and limed (or not) plots. After six months, we assessed the burrowing behavior and litter consumption of the earthworms. *L. terrestris* showed a good survival rate (>52%), as assessed by surface activity, in all situations. Liming had no detectable effect whereas the increase in bulk density in compacted plots (from 1.29 to 1.36 g cm^{-3}) led to a significant decrease in the burrow volume. This suggests that, when forced to do so, *L. terrestris* is able settle in compacted and acidic soils and then perform its functional role (burrow creation and litter burial).

Keywords: earthworm ; epi-anecic ; bulk density ; burrowing behaviour ; soil restoration

Soil functioning is now considered one of the pillars of environmental quality alongside water and air (Bünemann et al., 2018). Human activities have a significant impact on this ecosystem and it is sensitive to the numerous disturbances that are more frequently encountered in semi-natural environments such as forests (Roger-Estrade et al., 2011). Indeed, the increasing mechanization of forestry techniques and the use of increasingly heavier machinery on ever shorter rotations lead to problems of soil compaction and tree growth, and greatly contribute to the degradation of forest soils (Goutal, 2012; Solgi et al., 2020). This degradation, which may be more or less severe depending on the weight and number of passes of the machinery, depends strongly on soil texture (i.e. the size distribution of the mineral particles: clay, silt, sand) and the soil moisture (Pischedda, 2009; Naghdi et al., 2020). Forests with silty soils are particularly sensitive to compaction (Ranger et al., 2015), especially when forestry works are carried out on moist soil. Indeed, this can directly affect the physical fertility of the soil (Haynes and Naidu, 1998) by modifying the mechanical resistance of the aggregates (structural stability), increasing its apparent density (mass per unit volume of dry soil) and reducing macroporosity (porosity > 1 mm, visible to the naked eye) and the connectivity between pores (Zhai and Horn, 2019). These phenomena may subsequently lead to a decrease in tree root density (Von Wilpert and Schäffer, 2006), water infiltration and gas flow (Rabot et al., 2018). In addition, because the natural resilience of forest soils is generally low (Goutal et al., 2012), damage to these ecosystems may be irreversible.

Since natural regeneration of the soil is slow (Drewry, 2006) and because the timber industry is socio-economically important, solutions to maintain, preserve or restore the quality of forest soils are needed. Recently, prevention and advice tools for managers were developed (Pischedda, 2009) with the aim of promoting practices that prevent forest soil degradation while raising the awareness of stakeholders on this issue. However, settlement and compaction due to the passage of heavy machinery in some forest soils have already resulted in productivity losses. In these cases, restoration work can be carried out to restore functions of the ecosystem that has been degraded, increasingly used but this type of work remains difficult because of the stumps and roots and these methods can be expensive depending on the type of soil (Jabiol, 2013).

Several studies proposed that the properties of degraded agroecosystem soils can be restored using roots or soil organisms (Snyder and Hendrix, 2008; Pulido-Moncada et al., 2020) and more particularly the soil engineers, represented by the Lumbricidae, Formicidae and Isoptera (Jones et al., 1994; Jouquet et al., 2014). For example, earthworms (Lumbricidae) constantly modify their environment by burrowing galleries while ingesting soil and organic matter (Lavelle et al., 1997). They have an important role in many processes regulating soil structure, such as water infiltration and gas exchange (physical fertility), but also in the nutrient cycle and organic matter recycling (chemical fertility) (Bottinelli et al., 2015). In Europe, Lumbricidae have sometimes been used for soil restoration in ecological (or pedological) engineering because they are one of the organisms on which the most knowledge has been obtained (Butt, 2008) and several studies have shown their positive impact on soil properties (Jouquet et al., 2014). In fact, for the restoration to be effective, the selected organisms need to be well adapted to the environment. This requires a sound understanding of their biology and ecology. For example, most earthworm species are sensitive to acidic pH and are therefore found in low abundance in soils at pH < 5.5 (Satchell, 1955; Ammer et al., 2006). To remedy the acidity of forest soils and increase the activity and survival of earthworms, it is possible to apply $CaCO_3$ on nutrient-poor soils using the technique known as liming (Auclerc et al., 2013). This process is used on forest soils to regulate Ca and Mg deficiencies due to acid rain or excessive biomass removal that can deplete soils on certain types of nutrient-poor rocks (Court et al., 2018).

Despite the increasing problems of forest soil compaction and the growing accumulation of knowledge about earthworms, very few studies have actually assessed the suitability of earthworm inoculation to restore degraded forest soils. To the best of our knowledge, only Ampoorter et al. (2011) and Muys et al. (2003) have attempted to observe the effect of earthworm inoculations on was very low after one year or more. In agreement with current knowledge on the burrowing activity (bioturbation) and litter consumption of earthworms and frequency in forest soils, *Lumbricus terrestris,* an epi-anecic earthworm (Bottinelli et al., 2020) that creates deep vertical burrows, was often selected for soil restoration.

Before releasing earthworms in degraded forest soils, it is first necessary to evaluate whether they can survive in such an environment characterized by a double constraint, i.e. high soil bulk density and low pH. Thus, we first carried out a laboratory experiment to evaluate the burrowing capacity of *L. terrestris* in one acidic forest soil (pH= 4.4) with increasing soil bulk densities. Then, using cages (Baker et al., 1996), we released *L. terrestris* individuals in an experimental site where two different treatments (liming, compaction) were implemented ten years ago, alone or in combination (Bottinelli et al., 2014). We followed earthworm activities (organic matter burial, casting and burrowing activities) in order to estimate their potential as an agent of soil restoration.

2. Materials and methods

2.1. Study site and soil

The Grand Pays forest (49°8'27.59"N, 5°1'9.58"E) is located about 270 m above sea level near the town of Clermont-en-Argonne in the North-East of France. This is an experimental site managed by INRAE (National Research Institute for Agriculture, Food and the Environment) and ONF (Office National des Forêts), used to study the medium and long-term effects of moderate soil compaction in forest soils.

The soil at the site is a Luvisol (ruptic) (IUSS Working Group WRB, 2015) or Neoluvisol according to Baize et al. (2009). It is polycyclic and more acidic at the surface (water pH = 4.42) than at depth (pH of 5 between 0.7 to 1 m). An acidic mull humus is formed on a surface silt loam of about 50 cm which rests on deep clay from the Cenomanian gaize. The soil’s bulk density depth. In addition, the soil surface horizon is poorer in nutrient cations than the deep horizons which forces the roots to penetrate deeper to ensure good tree growth.

This 6-ha site, initially occupied by a mature stand of approximately 100-year-old beech (*Fagus*) and oak (*Quercus*) trees, was clearcut at the beginning of 2007. Logs were removed using a cable yarding system to avoid soil disturbance. The site was then divided into three blocks, and each block into four plots (30 m x 50 m) to study the two factors of compaction and liming. Each plot was thus randomly-assigned with one of the four following treatment groups: control (not compacted, not limed), compacted (but not limed), limed (but not compacted) and compacted and limed.

To create the compacted plots and study the recovery of the soil from compaction, a simulated trafficking event was carried out on the designated plots (March, 2008) using a forestry skidder (VALMET 8) with eight twin wheels in a charged cradle bogie (15 t empty and 20.5 t charged) driven over the whole surface of the plots. Lime was added to limit nutrient deficits. To create the limed plots, in September 2008, lime as powdered dolomite enriched with magnesium lime (CaO 36% and MgO 24%, at a calculated application rate of 630 kg ha^{-1} of CaO and 415 kg ha^{-1} of MgO) was applied on the designated plots. Potassium sulphate (K_2O: 50% and S: 17%, at 360 kg ha^{-1} of K_2O) was also added to avoid an imbalance between nutrients (Bonneau, 1995). After plots were prepared, a mono-specific plantation of sessile oak (*Quercus petraea* L.), which adapt easily to silty soils was planted in autumn of 2008 (and dead trees were replaced the following year) over the whole block. At this site, earthworm abundance was low and highly dominated by epigeic earthworms (*Lumbricus castaneus*) up to 2011 (Bottinelli et al., 2014).

2.2. Laboratory experiment

To evaluate the capacity of the earthworms to burrow vertical galleries in acidic soils under varying degrees of compaction, we used mesocosms (repacked soil cores) with increasing soil bulk densities

Pays” experimental site. Mesoscoms were transparent PMMA (methyl polymethacrylate) plastic cylinders 9.84 cm in diameter and 35 cm deep. The soil was thoroughly mixed, air dried for 4 days then sieved to 2 mm. Repacked soil cores were made with layers of 400 g of dry soil that were artificially compacted with a hydraulic press to obtain chosen soil thicknesses and thus soil BD. A total of 28 mesocosms was made with average BD of 1.30, 1.40, 1.50 and 1.60 g cm^{-3} (± 0.01) with 7 cores for each density level. One adult *L. terrestris* earthworm (mean (± SE) weight = 4.27 ± 0.27 g) was introduced per mesocosm and 2 g of litter (oak leaves also from the Grand Pays site) was added. The earthworms were bought at a local fishing store and kept in a bucket partly filled with the “Grand Pays” soil and some litter for several weeks for acclimation. The mesocosms were kept under constant conditions at 20°C with natural night and day cycles for four weeks.

2.3. Field experiment

In one block at the site, we selected three subplots (1 m x 4 m) for each of the four treatments, thus 12 subplots in total. In each subplot, six PVC cylinders (10 cm diameter and 15 cm length) were gently inserted into the wet soil using a wood hammer until 5 cm of PVC remained above the soil. In each subplot, two mesocosms were kept as controls without earthworms and four were inoculated with one adult *L. terrestris* (mean (± SE) weight = 4.10 ± 0.32 g). Natural litter was removed from the soil surface. Four grams of oak litter leaves was added in all the mesocosms. Each mesocosm had a lid comprising a stainless-steel 3-mm-opening mesh to prevent earthworms from escaping. In total, 72 mesocosms were implanted in the soil at the experimental plot, 48 of them with earthworms and 24 controls without earthworms.

Every month (from November 2017 to April 2018), we visually checked the quantity of litter remaining in all mesocosms. If there was little litter remaining, 4 g of leaves were added at the soil surface. The rate of litter disappearance was used as a proxy for assessing earthworm survival.

length) around the mesocosms to sample a soil column going deeper into the soil and containing the mesocosm and thus the putative earthworm burrows. On the same day, twenty mL of chloroform was poured into each mesocosm in order to kill the earthworms therein. We collected a soil sample (0-30 cm depth) next to each mesocosm using a 2-cm diameter stainless-steel gouge auger to assess soil water content profiles. The samples were cut into six layers with a 5 cm increment.

2.4. Physical and chemical measurements

The soils columns (from the laboratory experiment or sampled in the field) were scanned using an X-ray scanner (50 mA, 120 kV) to obtain sets of 16 bits images. Images were analyzed using ImageJ (Schindelin et al., 2012) and BoneJ (Doube et al., 2010). First images were segmented and then the largest macropores (volume higher than 5 cm^3) were selected to obtain earthworm burrows since *L. terrestris* is known to build large and continuous burrows (Jégou et al., 1999; Dittbrenner et al., 2011). Once the main burrows were selected, we improved the results manually in ImageJ by either removing some parts of the burrow that were obviously not made by earthworms (for example cracks or tiny cylindrical burrows) and adding other parts that were disconnected from the main burrow by, for example, casts filling the burrows as could occur for lateral branches.

The objectives were (i) to determine if the earthworms settled in the soil core (based on the presence / absence of a large burrow) and (ii) to measure the main characteristics of the *L. terrestris* burrows: volume, diameter, maximal depth, percentage of the burrow volume in the first 5 cm. This latter parameter was computed to take into account some of the results obtained in the laboratory experiments (sub-horizontal burrows).

X-ray images from medical scanners are automatically calibrated in Hounsfield values and also provided information on the BD of the (wet) soil. For field mesocosms, these wet BD were estimated for different depths (0-5, 5-10, 10-15 and 15-20 cm) in the images using the modes of the finally obtain estimates of the soil (dry) BD.

In each field mesocosm, the remaining litter was collected, cleaned to remove mineral particles, dried in an oven for 72 hours at 40 °C, and weighed. We calculated litter consumption in each mesocosm during the field experiment and conjectured that in earthworm-free mesocosms, the consumption was due to microorganisms or other small invertebrates. After tomography and destruction of the mesocosms, pH was measured within each field mesocosm at two depths (0-10 cm and 15-25 cm) following ISO 10390 (2005).

2.5. Statistical analysis

For the parameters associated with the laboratory experiments (burrow characteristics, cast production and litter disappearance), we used a one-way ANOVA with soil BD as a factor. In all cases, data were log-transformed to reach homoscedasticity (assessed with a Bartlett test). For burrow characteristics in the field experiment, we used a two-way ANOVA with liming and compaction as factors. For soil BD and pH in the field experiment, we used a resampling test for each factor separately using the 'coin' package. In this latter case, the interaction between the two factors could not be computed and was only assessed visually with interaction plots. All computations were carried out in R v3.3.3 (R core team 2019).

3. Results

3.1. Laboratory experiment

A few representative examples of 3D burrow reconstructions are shown in Fig. 1. Soil BD and thus compaction had a strong and significant effect on *L. terrestris* burrowing under laboratory conditions. The volume of the burrows made by each individual decreased, albeit non significantly, when the BDs increased from 1.3 to 1.4 and 1.5 g cm^{-3} (Fig. 2). However, the burrow volume greatly and significantly decreased (-95%) at a BD of 1.6 g cm^{-3} compared to the other tested BD but simply remained just below the soil surface. The diameter of the burrow followed the same trend (p-value < 0.01; $F_{3,24} = 5.3$; ANOVA) and was significantly lower at 1.6 than at 1.3 g cm^{-3} (-50%; Fig. 3). The diameter for the mid-range BD was very variable and thus not significantly different from the highest and lowest tested BD. The maximum burrow depth was significantly greater at 1.3 than at 1.5 g cm^{-3} with a decrease of approximately 65% (p-value < 0.0001; $F_{3,24} = 22.2$; ANOVA; Fig. 4). At 1.4 g cm^{-3}, the maximal burrow depth was intermediate and not significantly different from those observed at 1.3 and 1.5 g cm^{-3} due to a high variability. At the highest BD, the burrow depth was very limited and significantly different from all other tested BD.

BD also had an impact on the surface behavior of *L. terrestris* with a significant decrease in surface cast production (p-value = 0.015; $F_{3,24} = 4.3$; ANOVA) and organic matter burial (p-value < 0.0001; $F_{3,24} = 26.4$; ANOVA). Cast production was significantly lower at 1.5 and 1.6 g cm^{-3} (on average -60%) than at 1.3 g cm^{-3} (Table 1). The percentage of consumed or buried organic matter was significantly lower only at 1.6 g cm^{-3} compared to the other tested BDs.

3.2. Field experiment

Ten years after the compaction event, we still observed a significantly higher soil BD for the 5-10 cm layer in the compacted plots (p-value < 0.001; Z = -3.8; resampling test; Table 2). Plots that were limed showed a significantly lower BD compared to non-limed plots (p-value = 0.036; Z = -2.1; resampling test). For the deeper soil layer (15-20 cm), no difference was observed between compacted and non-compacted plots but there was still a significant effect of liming with higher BD in non-limed plots (p-value = 0.009; Z = -2.6; resampling test).

Regarding pH, liming caused a significant increase at both depths considered (0-10 and 20-30 cm; p-value = 0.02; Z = 2.3 and p-value = 0.003; Z = 2.9, respectively; resampling tests). Moreover, non-compacted plots also had significantly higher pH values than compacted plots at tests).

For pH and soil BD, we used randomization tests and thus we could not test the effect of an interaction between factors (liming and compaction). We thus only visually verified that no obvious or significant interactions were present using graphical analysis (interaction plots).

The rate of litter disappearance at the soil surface in the cores, decreased with time (Fig. S1) but indicated that earthworms were active in the cores and thus alive. There were no obvious differences between treatments except after 5 months. At this date, activity seemed to be higher in the non-limed plots.

Typical examples of 3D burrow reconstructions are shown in Fig. 5. Earthworm burrow systems made by *L. terrestris* in the PVC cores were not very different among the treatment plots. No differences were observed for diameter, the burrow volume in the 0-5 cm layer and the maximal burrow depth (Table 2). This latter parameter was however influenced by our experimental design and the limited depth of the PVC cores. The only significant difference was observed for burrow volume which was significantly influenced (and reduced) due to compaction (p-value = 0.003; $F_{1,43}$ = 11.5; ANOVA). The decrease was approximately 40% between compacted and non-compacted plots.

The absence or presence of earthworms in the PVC cores had a significant effect (p-value < 0.001; $F_{1,61}$ = 35.4; ANOVA) on the amount of litter buried or consumed (1.87 ± 0.55 vs 6.21 ± 0.29 g (mean ± SE) respectively in six months) but neither compaction or liming had a significant effect on the amount of litter buried or consumed (when only cores with earthworms were considered; Table 2).

4. Discussion

4.1. Effects of compaction on *L. terrestris* behavior

laboratory conditions with a larger range of BDs tested, burrow volume, diameter and maximal depth were negatively impacted aa was cast production and litter consumption. Under natural conditions with a much smaller variation in BD between compacted and uncompacted treatments, only burrow volume was impacted. In the experimental site, ten years after the compaction, the bulk densities were still higher (+ 0.07 g cm^{-3}) in the compacted plots compared to the control plots but this difference was limited compared to that found immediately after the compaction (+ 0.09 g cm^{-3}; Goutal et al., 2012).

It was already well known that *L. terrestris* burrowing behavior is sensitive to soil compaction. However, past studies either used a range of BD (from 1.47 to 1.68 g cm^{-3}) but no replicates (Rushton, 1986) or only two very contrasting soil BDs, i.e. compacted vs non-compacted soil (Joschko et al., 1993; Jégou et al., 2002; Müller-Inkmann et al., 2013). Moreover, it is impossible to compare our results with those of Rushton (1986) since this author did not describe the texture of the soil used, and so BDs cannot be corrected and compared (Beylich et al., 2010; Keller and Hakasson, 2010). Also due to the inherent variability linked to behavior in general, the linear relationships between burrow length and BD in the study of Rushton (1986) was significant but low (linear regression: $p = 0.020$, $R^2 = 0.34$). However our findings were comparable with those of Pöhlitz et al. (2020) who showed that, for silt loam soils, the number of biopores produced by *L. terrestris* decreased between 1.35 and 1.6 g cm^{-3} with about 30% less biopores at 1.6 g cm^{-3} (albeit this decrease was not significant). Moreover, the relationships between biopore number and BD were not linear (cf Fig. 5C in Pöhlitz et al., 2020), which is in agreement with our findings, also in a silt loam soil. It appeared that for the earthworm species *L. terrestris,* there is a threshold effect. Below a given BD value, *L. terrestris* tend to build a typical deep vertical burrow (even if the trend is a gentle decrease with increasing BDs) since this burrow is also a crucial shelter. Above this BD value (between 1.5 and 1.6 g cm^{-3} in our case) they produce a very short and not necessarily vertical since we observed a high variability of response for maximal burrow depth for this BD.

In contrast to many previous laboratories studies (as reviewed by Beylich et al., 2010), we did not observe an increase in surface cast production with increasing BDs but instead a clear decrease with a significant difference between 1.3 and 1.5 g cm^{-3}. There are two contradicting phenomena which could explain this observation and thus the resulting outcome is difficult to predict. First, most authors, as reviewed by Beylich et al. (2010), suggested that *L. terrestris* do cast more at the soil surface in compacted soils since there is less free space within the soil and they do not want to refill or obstruct their main burrow. These authors assumed that due to energy cost, in compacted soils *L. terrestris* no longer burrowed small lateral dead-end burrows that were sometimes observed (Grigoropoulou et al., 2008) and that could be used for casting. Second, from a purely physical point of view, if the burrow volume decreases in compacted soil then the volume of casts produced should also decrease, even if the earthworm is obliged to ingest the soil to burrow when BD are high (Dexter, 1978).

Another observation from our laboratory experiment was the trend for a decrease in diameter of the main burrow, suggesting soil compaction had an effect on *L. terrestris* behavior. A compacted zone of a few mm width was previously observed around the burrows of this species as its crushes its own casts against the burrow walls (Rogasik et al., 2014). Besides these so-called ‘cutanes’, it is thus likely that the daily reuse of the vertical burrow forces this earthworm to use its hydraulic skeleton to anchor its body to crawl up which simply exerts a repeated lateral pressure on the burrow walls. If the burrow diameter decreased when soil BD increased, it could mean that the earthworms were less active or that the lateral pressure has a lesser impact when applied to a soil matrix that is already compacted.

The most striking finding from our laboratory experiment was the constant decrease in burrow maximal depth with increasing BDs. This result appears surprising considering that *L. terrestris* is believed to live deep in the soil while our mesocosms were only 30 cm depth, so more than 1 m according to Shipitalo and Butt (1999), was observed for agricultural croplands only. This view was further reinforced by laboratory studies where this earthworm was introduced into mesocosms of 30 to 40 cm depth and filled with agricultural or fallow land soils (Jégou et al., 1999; Langmaack et al., 1999; Dittbrenner et al., 2011; Crumsey et al., 2013; Capowiez et al., 2015). In all cases, the *L. terrestris* burrows always reached the bottom of the soil cores. However, *L. terrestris* is also known to exhibit behavioral plasticity (Lavelle et al., 1983 cited by Lee, 1985). Compared to agricultural soils, forest soils have more abundant litter and do not get as dry during summer. Hence, in forest soils, it is likely that *L. terrestris* does not need to burrow as deep. But even in agricultural soils, there are some observations showing that this species do not always burrow very deeply or only vertically either under field conditions (Nuutinen et al., 2017) or in laboratory conditions (Evans, 1947; Bastardie et al., 2005; Rogasik et al., 2014). Overall, the plasticity of *L. terrestris* could be triggered by organic matter quantity and location (Evans, 1947; Frazao et al., 2019) or soil pollution (Mombo et al., 2018) leading to modification of the archetypical burrow (vertical, deep and rarely branched) of this species. This suggests that this species is not a truly anecic species (sensus Bouché, 1972) but rather an epi-anecic species, able to shift from one ecological category to another (sometimes epigeic in forest soils to typically anecic in agricultural soils). In their recent study aiming at better defining the ecological categories, Bottinelli et al. (2020) determined that this species (called *L. herculeus* by Bouché, 1972, see James et al., 2010, for more details) is 66% anecic and 34% epigeic.

In brief, the effects of compaction were clearly observed during the laboratory experiment whereas compaction appears to have only an impact on burrow volume under our field conditions. The values of BD and the difference in BD between the compacted and non-compacted plots in the field experiment were both low (1.36 vs 1.29 g cm^{-3}). Indeed effects were observed under laboratory conditions for much higher BDs (1.5 or 1.6 g cm^{-3} depending on the studied parameters).

Ten years after the experiment was set up, the impact of liming was still observable on the surface horizon (0-10 cm) with a higher pH for the limed treatment groups. This was also true for the soil BD, which remained higher in the compacted plots. Although the soil has partially restored itself naturally on the surface, it had not returned to its initial state (i.e. the control plot). This further confirms that forest soil regeneration by natural actors (climate, soil organisms and roots) is slower than for agricultural soils on which more management approaches (such as seed dressing or tillage) are carried out every year (Capowiez et al., 2012; Bottinelli et al., 2014). It is then valid to attempt to help restoration using physical methods such as mulching for example or by stimulating or introducing soil ecosystem engineers such as earthworms and in particular anecic or epi-anecic ones. This approach for ecological restoration was previously reported but, in both cases, the released anecics (including *L. terrestris*) did not survive (Muys et al., 2003) or were almost not retraced (Ampoorter et al., 2011), even though *L. terrestris* is often thought to be more acido-tolerant than other anecics (Muys et al., 2003). Indeed the inherent difficulties of this kind of approach should not be minimized: if few or no earthworms are initially found in the degraded (compacted) zones, this suggests that these conditions cannot support their settlement and survival and that they preferred to migrate. Generally distance migration for earthworms is low (Eijsackers, 2011), although *L. terrestris* is a peregrine species with higher colonization capacities and is able to crawl up to 20 m in one night at the soil surface (Lee, 1985). It appears that the choice of releasing *L. terrestris* is paradoxical as recent attempts to release this earthworm even in agricultural plots, *a priori* more favorable, were proven to be slow (Nuutinen et al., 2017). These authors observed that *L. terrestris* managed to settle in wheat plots but only ten years after initial release and even so the mean abundance remained low (less than 5 individual m^{-2}).

Conclusions

very low. Under our experimental conditions (encaged earthworms), *L. terrestris* survived well for six months even in degraded soils (compacted and non-limed), as assessed by their feeding and burrowing activities. However we did not determine whether they would reproduce. Further studies are needed to examine whether young earthworms, with different behavior and preferred depth, could survive under these conditions. Releasing anecic earthworms is often thought to be an effective first step in soil remediation since these earthworms buried a lot of organic matter and create large vertical burrows that will enable later the settlement of endogeic earthworms, the intensive bioturbators of the topsoil layer. However the choice of *L. terrestris* as an effective restoration agent should remain an open question since its behavioral plasticity and slow settlement speed could be seen as possible limiting factors (Butt, 2008).

List of references

Ammer, S., Weber, K., Abs, C., Ammer, C., Prietzel, J., 2006. Factors influencing the distribution and abundance of earthworm communities in pure and converted Scots pine stands. Appl. Soil Ecol. 33, 10–21.

Ampoorter, E., De Schrijver, A., De Frenne, P., Hermy, M., Verheyen, K., 2011. Experimental assessment of ecological restoration options for compacted forest soils. Ecol. Eng. 37, 1734–1746.

Auclerc, A., Capowiez, Y., Guérold, F., Nahmani, J., 2013. Application of X-ray tomography to evaluate liming impact on earthworm burrowing activity in an acidic forest soil under laboratory conditions. Geoderma 202–203, 45–50.

Baize, D., Girard, M., AFES, 2009. Référentiel pédologique 2008, Éditions Quæ, Versailles.

Baker, G.H., Barrett, V.J., Carter, P.J., Woods, J.P., 1996. Method for caging earthworms for use in field experiments. Soil Biol. Biochem. 28, 331–339.

Bastardie, F., Capowiez, Y., Renault, P., Cluzeau, D., 2005. A radio-labelled study of earthworm behaviour in artificial soil cores in term of eco- logical types. Biol. Fertil. Soils 41, 320–327.

effects on soil biota and soil biological processes in soils. Soil Till. Res. 109, 133–143.

Bonneau, M., 1995. Fertilisation des forêts dans les pays tempérés: théorie, bases du diagnostic, conseils pratiques, réalisations expérimentales. École Nationale du Génie Rural et des Forêts, Nancy, PhD dissertation.

Bottinelli, N., Capowiez, Y., Ranger, J., 2014. Slow recovery of earthworm populations after heavy traffic in two forest soils in northern France. Appl. Soil Ecol. 73, 130–133.

Bottinelli, N., Hedde, M., Jouquet, P., Capowiez, Y., 2020. An explicit definition of earthworm ecological categories - Marcel Bouché's triangle revisited. Geoderma 372, 114361.

Bottinelli, N., Jouquet, P., Capowiez, Y., Podwojewski, P., Grimaldi, M., Peng, X., 2015. Why is the influence of soil macrofauna on soil structure only considered by soil ecologists? Soil Till. Res. 146, 118–124.

Bouché, M.B., 1972. Lombriciens de France : Écologie et systématique, INRA, Paris.

Bullock, J.M., Aronson, J., Newton, A.C., Pywell, R.F., Rey-Benayas, J.M., 2011. Restoration of ecosystem services and biodiversity: conflicts and opportunities. Trends Ecol. Evol. 26, 541–549.

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., Pulleman, M., Sukkel, W., van Groenigen, J.W., Brussaard, L., 2018. Soil quality – A critical review. Soil Biol. Biochem. 120, 105–125.

Butt, K.R., 2008. Earthworms in Soil Restoration: Lessons Learned from United Kingdom Case Studies of Land Reclamation. Restor. Ecol. 16, 637–641.

Capowiez, Y., Bottinelli, N., Sammartino, S., Michel, E., Jouquet, P., 2015. Morphological and functional characterisation of the burrow systems of six earthworm species (Lumbricidae). Biol. Fertil. Soils 51, 869–877.

Capowiez, Y., Sammartino, S., Cadoux, S., Bouchant, P., Richard, G., Boizard, H., 2012. Role of earthworm in regenerating soil structure after compaction in reduced tillage systems. Soil Biol. Biochem. 55, 93–103.

-

Long-term effects of forest liming on mineral soil, organic layer and foliage chemistry: Insights from multiple beech experimental sites in Northern France. For. Ecol. Manag. 409, 872–889.

Crumsey, J., Le Moine, J.M., Capowiez, Y., Goodsitt, M.M., Larson, S.C., Kling, G.W., Nadelhoffer, K.J., 2013. Community-specific impacts of exotic earthworm invasions on soil carbon dynamics in a sandy temperate forest. Ecology 94, 2827–2837.

Dexter, A.R., 1978. Tunnelling in soil by earthworms. Soil Biol. Biochem. 10, 447–449.

Dittbrenner, N., Moser, I., Triebskorn, R., Capowiez, Y., 2011. Assessment of short and long-term effects of imidacloprid on the burrowing behaviour of two earthworm species (*Aporrectodea caliginosa* and *Lumbricus terrestris*) by using 2D and 3D post-exposure techniques. Chemosphere 84, 1349–1355.

Doube, M., Kłosowski, M.M., Arganda-Carreras, I., Cordelières, F.P., Dougherty, R.P., Jackson, J.S., Schmid, B., Hutchinson, J.R., Shefelbine, S.J., 2010. BoneJ: Free and extensible bone image analysis in ImageJ. Bone 47, 1076–1079.

Drewry, J.J., 2006. Natural recovery of soil physical properties from treading damage of pastoral soils in New Zealand and Australia: a review. Agric. Ecosyst. Environ. 114, 159–169.

Evans, A.C., 1947. A method for studying the burrowing activities of earthworms. Ann. Mag. Nat. Hist. 11, 643–650.

Eijsackers, H., 2011. Earthworms as colonizers of natural and cultivated soil environments. Appl. Soil Ecol. 50, 1–13.

Frazao, J., de Goede, R., Capowiez, Y., Pulleman, M., 2019. Soil structure formation and organic matter distribution as affected by earthworm species interaction and crop residue placement. Geoderma 338, 453–463.

Goutal, N., 2012. Changes and recovery in soil physical and chemical characteristics of two forest soils following traffic by a full-loaded forwarder. AgroParisTech, Paris, PhD dissertation.

Goutal, N., Boivin, P., Ranger, J., 2012. Assessment of the natural recovery rate of soil specific volume following forest soil compaction. Soil Sci. Soc. Am. J. 76, 1426–1435.

hatchtings in Evans' boxes. Pedobiologia 51, 343–349.

Haynes, R., Naidu, R., 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. Nutrient Cycling in Agroecosystems 51, 123–137.

ISO 10390, 2005. Soil quality – Determination of pH, second ed. International Organization for Standardization, Geneva, Switzerland.

IUSS Working Group WRB, 2015. World Reference Base for Soil Resources 2014, update 2015. International soil classification system for naming soils and creating legends for soil maps, World Soil Resources, Reports No. 106, FAO, Rome.

Jabiol, B., 2013. La structure des sols forestiers, spécificités, états, conséquences et enjeux, in: Baize D. et al. (Eds.), Les sols et leurs structures: observations à différentes échelles, Quae, Versailles, pp. 79–92.

James, S.W., Porco, D., Decaëns, T., Richard, B., Rougerie, R., Erseus, C., 2010. DNA barcoding reveals cryptic diversity in *Lumbricus terrestris* L., 1758 (Clitellata): ressurection of *L. herculeus*. Plos One 12, e15629.

Jégou, D., Hallaire, V., Cluzeau, D., Tréhen, P., 1999. Characterization of the burrow system of the earthworms *Lumbricus terrestris* and *Aporrectodea giardi* using X-ray tomography and image analysis. Biol. Fertil. Soils 29, 314–318.

Jégou, D., Brunotte, J., Rogasik, H., Capowiez, Y., Diestel, H., Joschko, M., Schrader, S., 2002. Impact of soil compaction on earthworm burrow systems using X-ray computed tomography: preliminary study. Eur. J. Soil Biol. 38, 329–336.

Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. Oikos 69, 373–386.

Joschko, M., Muller, P.C., Kotzke, K., Dohring, W., Larink, O., 1993. Earthworm burrow system development assessed by means of X-ray computed tomography. Geoderma 56, 209–221

Jouquet, P., Blanchart, E., Capowiez, Y., 2014. Utilization of earthworms and termites for the restoration of ecosystem functioning. Appl. Soil Ecol. 73, 34–40.

soil organic matter content. Geoderma 154, 398–406.

Langmaack, M., Schrader, S., Rapp-Bernhardt, U., Kotzke, K., 1999. Quantitative analysis of earthworm burrow systems with respect to biological soil-structure regeneration after soil compaction. Biol. Fertil. Soils 28, 219–222.

Lavelle, P., Zaidi, Z., Schaefer, R., 1983. Interactions between earthworms, soil organic matter and microflora nan African savanna soil, in: Lebrun P. et al. (Eds), New trends in soil biology, Proceedings of the 8th International Colloquium of Soil Zoology, Louvain la neuve, pp. 253–259.

Lavelle, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Ineson, P., Heal, O., Dhillion, S., 1997. Soil function in a changing world: the role of invertebrate ecosystem engineers. Eur. J. Soil Biol. 33, 159–193.

Lee, K.E., 1985. Earthworms their ecology and relationships with soils and land use, Academic Press, Sydney.

Mombo, S., Laplanche, C., Besson, P., Sammartino, S., Schreck, E., Dumat, C., Capowiez, Y., 2018. Metal soil pollution differentially affects both the behaviour and exposure of *A. caliginosa* and *L. terrestris*. Biol. Fertil. Soils 54, 319–328.

Müller-Inkmann, M., Fründ, H.C., Hemker, O., 2013. An experimental setup to assess earthworm behaviour in compacted soil. Biol. Fertil. Soils 49, 363–366.

Muys, B., Beckers, G., Nachtergale, L., Lust, N., Merckx, R., Granval, P., 2003. Medium-term evaluation of a forest soil restoration trial combining tree species change, fertilisation and earthworm introduction. Pedobiologia 47, 772–783.

Naghdi, R., Solgi, A., Labelle, E.R., Nikooy, M., 2020. Combined effects of soil texture and machine operating trail gradient on changes in forest soil physical properties during ground-based skidding. Pedosphere 30, 508–516.

the settlement of a semi-sedentary earthworm *Lumbricus terrestris* in an arable clay field. Soil Biol. Biochem. 115, 285–296.

Pischedda, D., 2009. Pour une exploitation forestière respectueuse des sols et de la forêt “Prosol”, FCBA and Office national des forêts, Paris.

Pöhlitz, J., Rucknagel, J., Schlütter, S., Vogel, H.J., Christen, O., 2020. Computed tomography as an extension of classical methods in the analysis of soil compaction, exemplified on samples from two tillage treatments and at two moisture tensions. Geoderma 346, 52–62.

Pulido-Moncada, M., Katuwal, S., Ren, L., Cornelis, W., Munkholm, M., 2020. Impact of potential bio-subsoilers on pore network of a severely compacted subsoil. Geoderma 363, 114154.

R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Rabot, E., Wiesmeier, M., Schlüter, S., Vogel, H.J., 2018. Soil structure as an indicator of soil functions: A review. Geoderma 314, 122–137.

Ranger, J., Goutal-Pousse, N., Bonnaud, P., Bedel, L., Davesne, R., Demainson, J., Nourrisson, G., 2015. Effect of silviculture mechanization on the quality of forest soils: Physical properties dynamics for natural and assisted revegetations, INRAE Grand-Est, Nancy.

Roger-Estrade, J., Adamiade, V., Arrouays, D., Baranger, E., Bartoli, M., Boizard, H., Brêthes, A., Brisson, N., Capowiez, Y., Chanzy, A., Chaplain, V., Cousin, I., Cosenza, P., Cui, K., Debuisson, S., Défossez, P., Gérard F., Jayet, P.-A., Labreuche, J., Le Bas, C., Lefevre Y., Leonard, J., Lévêque, E, Leveque F., Mary, B., Mumen, M., Ranger, J., Tabbagh, A., Tang, A.-M., Tessier, D., Richard, G., 2011. Dégradation physique des sols agricoles et forestiers liée au tassement : principaux résultats du projet GESSOL-ADD DST. Étude et Gestion des Sols 18, 187–199.

Rogasik, H., Schrader, S., Onasch, I., Kiesel, J., Gerke, H.H., 2014. Micro-scale dry bulk density variation around earthworm (*Lumbricus terrestris* L.) burrows based on X-ray computed tomography. Geoderma 213, 471–477.

populations on land reclaimed from open-cast coal mining. Pedobiologia 29, 85–90.

Satchell, J.E., 1955. Some aspects of earthworm ecology, in: Kevan, D.K.Mc.E. (Ed.), Butterworths Soil Zoology, London, pp. 180–201.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682.

Shipitalo, M.J., Butt, K.R., 1999. Occupancy and geometrical properties of *Lumbricus terrestris* L. burrows affecting infiltration. Pedobiologia 43, 782–794.

Snyder, B.A., Hendrix, P.F., 2008. Current and potential roles of soil macroinvertebrates (earthworms, millipedes, and isopods) in ecological restoration. Restor. Ecol. 16, 629–636.

Solgi, A., Najafi, A., Page-Dumroese, D.S., Zenner, E.K., 2020. Assessment of topsoil disturbance caused by different skidding machine types beyond the margins of the machine operating trail. Geoderma 367, 114238.

Von Wilpert, K., Schäffer, J., 2006. Ecological effects of soil compaction and initial recovery dynamics: a preliminary study. Eur. J. For. Res. 125, 129–138.

Zhai, X., Horn, R., 2019. Dynamics of pore functions and gas transport parameters in artificially ameliorated soil due to static and cyclic loading. Geoderma 337, 300–310.

Fig. 1. Examples of 3D reconstructions of *L. terrestris* burrow systems in repacked soil cores (25 cm in length and 16 cm in diameter) with increasing soil bulk densities. Burrow diameter cannot be directly compared in these images since it depends on whether the burrow is on the foreground or the background of the image.

Fig. 2. Box and whisker plots of burrow volume created by *L. terrestris* in repacked soil cores with four soil bulk densities. Boxes bearing different letters are significantly different from each other (ANOVA).

Fig. 3. Box and whisker plots of the diameter of *L. terrestris* burrows in repacked soil cores with four soil bulk densities. Boxes bearing different letters are significantly different from each other (ANOVA).

Fig. 4. Box and whisker plots of the maximal depth of *L. terrestris* burrow in repacked soil cores with four soil bulk densities. Boxes bearing different letters are significantly different from each other (ANOVA).

Fig. 5. Examples of 3D reconstructions of the *L. terrestris* burrow systems in caged mesocosms (30 cm in length and 16 cm in diameter) set-up at the 10-year old experimental site in which compaction and liming were tested.

consumed (means and SE) as a function of the soil bulk density for *L. terrestris* under laboratory conditions. Values bearing different letters are different at the 5% level (one-way anova).

Bulk density (g cm^{-3})	1.30	1.40	1.50	1.60
Amount of surface casts (g)	23.38^{a} (0.93)	13.90^{ab} (0.77)	9.31^{b} (0.83)	9.67^{b} (0.94)
Litter consumed or buried (%)	85.06^{a} (1.48)	86.95^{a} (1.28)	74.24^{a} (1.01)	42.11^{b} (0.91)

tests of the behaviour of *L. terrestris* in mesocosms under field conditions depending on compaction and liming. Values (in bold) bearing different letters are different at the 5% theshold level.

	Compacted	Non compacted	Limed	Non limed	Effect of compaction	Effect of liming	Interaction
Soil physico-chemical parameters (resampling test)							
Bulk density at 5-10 cm depth (g cm^3)	**1.36**^{a} (0.727	**1.29**^{b} (0.27)	**1.30**^{b} (0.84)	**1.34**^{a} (0.90)	P<0.001	P=0.036	-
Bulk density at 15-20 cm depth (g cm^3)	1.44 (0.28)	1.41 (0.28)	**1.39**^{b} (0.28)	**1.46**^{a} (0.29)	P=0.28	P=0.009	-
pH (water) at 0-10 cm depth	**4.81**^{b} (0.52)	**4.94**^{a} (0.52)	**5.05**$^{\mathbf{a}}$ (0.53)	**4.71**$^{\mathbf{b}}$ (0.51)	P=0.019	P<0.001	-
pH (water) at 20-30 cm depth	**4.67**^{b} (0.51)	**4.78**^{a} (0.52)	**5.77**^{a} (0.51)	**4.68**^{b} (0.51)	P=0.003	P=0.01	-
Characteristics of the earthworm behaviour (two-way anova)							
Volume of burrows (cm^3)	**9.51**^{b} (0.75)	**15.95**^{a} (0.97)	11.99 (0.84)	13.95 (0.90)	P=0.003	P=0.32	P=0.71

(mm)			(0.70	(0.69)			
Maximum burrow depth (cm)	12.51 (3.2)	14.32 (3.5)	13.39 (3.3)	13.52 (3.4)	P=0.11	P=0.84	P=0.48
Percentage of macroporosity in the first 5 cm depth (%)	32.80 (1.39)	29.21 (1.31)	33.25 (1.40)	29.05 (1.31)	P=0.44	P=0.19	P=0.57
Litter consumed or buried (g)	6.32 (0.73)	6.10 (0.71)	5.43 (0.67)	7.00 (0.76)	P=0.81	P=0.09	P=0.044

X The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

- Earthworm feeding and burrowing behavior was studied with increased soil bulk density
- Above soil bulk density of 1.4 g cm^{-3}, *Lumbricus terrestris* activity was significantly reduced
- Caged *L. terrestris* remained active in acidic compacted forest soils for 6 months
- Earthworms, as ecosystem engineers, can help to restore degraded forest soils

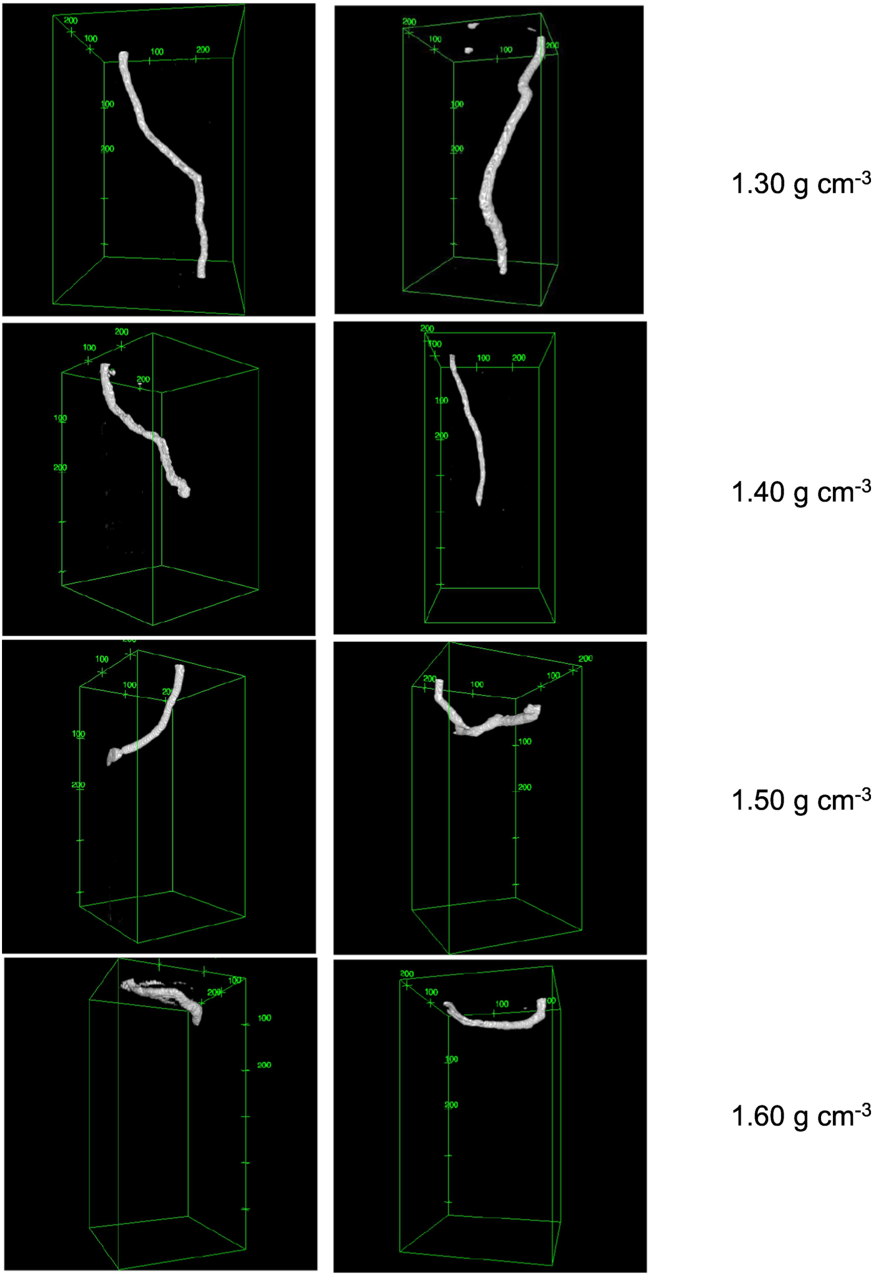
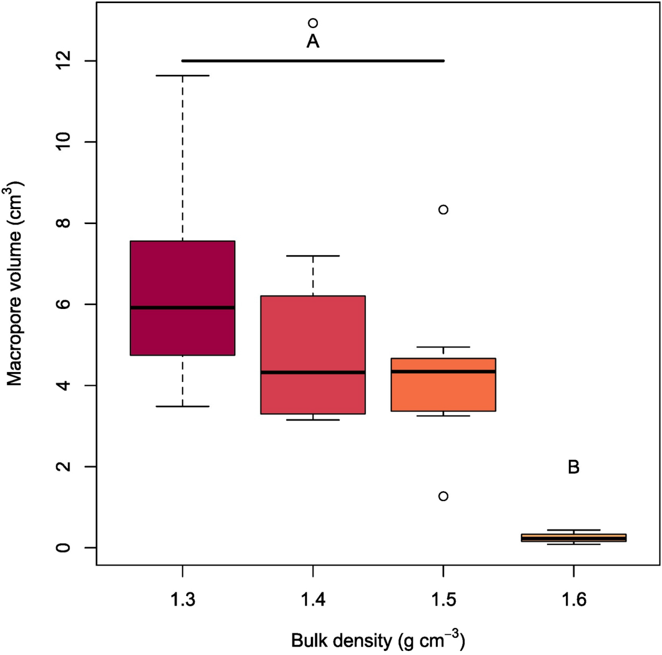
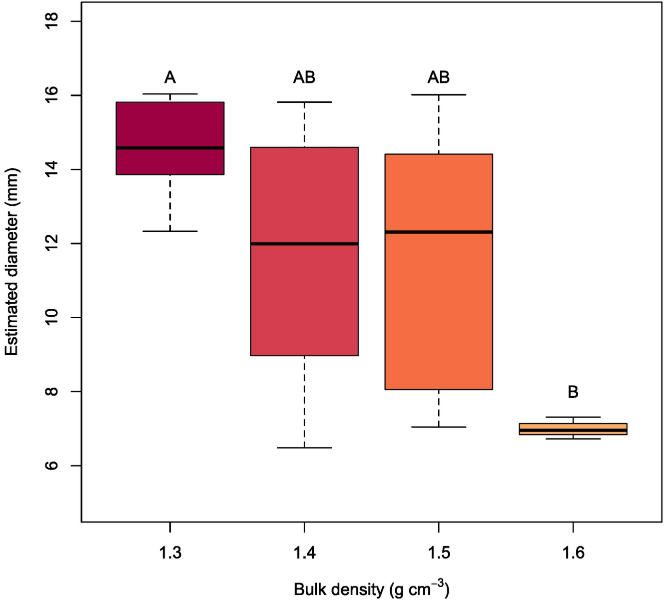


Figure 1



A
B
12
10
8
6
4
2
0
Macropore volume (cm^3)
1.3
1.4
1.5
1.6
Bulk density (g cm^{-3})

Figure 2



A
AB
AB
B
18
16
14
12
10
8
6
Estimated diameter (mm)
1.3
1.4
1.5
1.6
Bulk density (g cm^{-3})

Figure 3

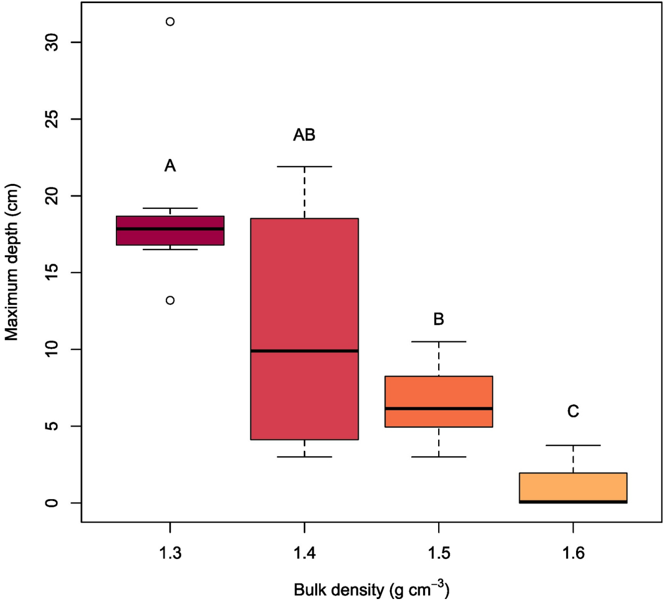

A
AB
B
C
0
5
10
15
20
25
30
Maximum depth (cm)
1.3
1.4
1.5
1.6
Bulk density (g cm^{-3})

Figure 4

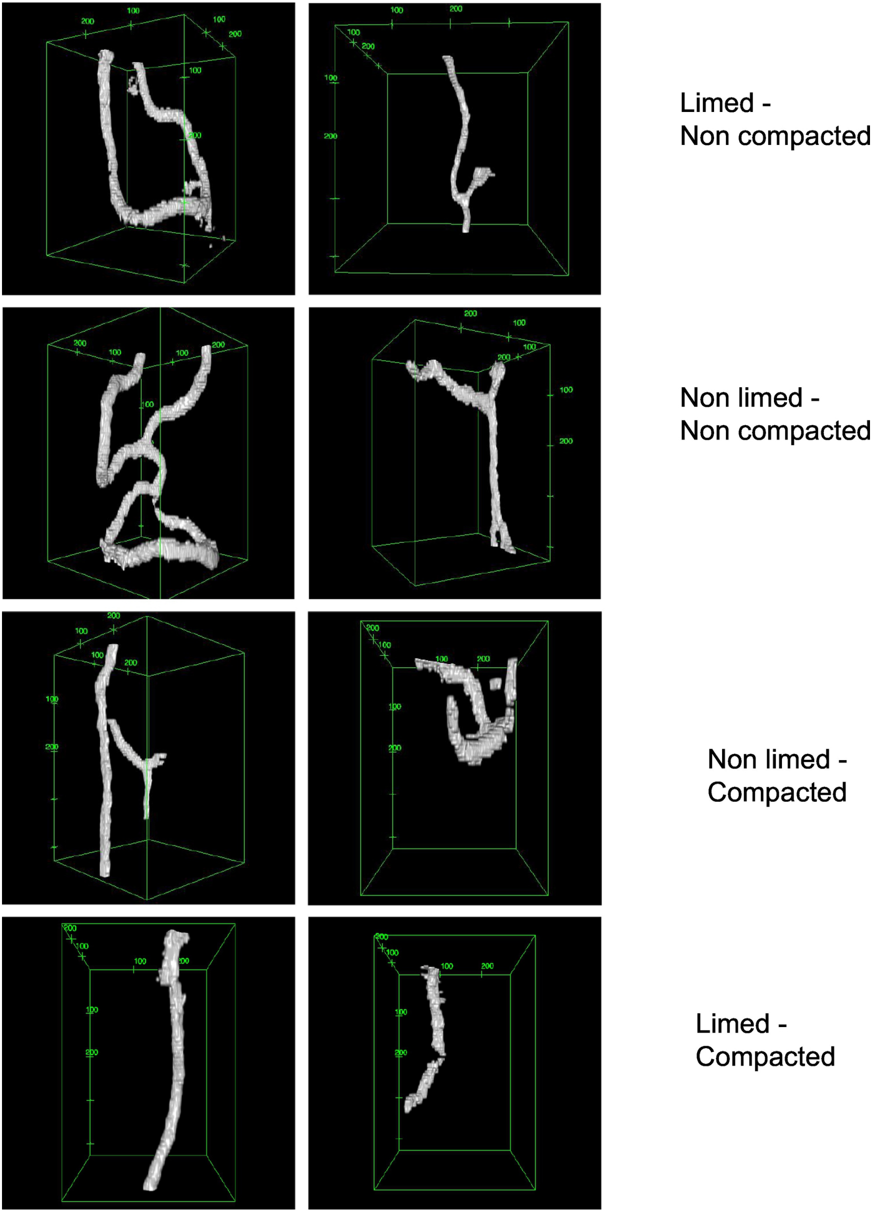

Limed - Non compacted
Non limed - Non compacted
Non limed - Compacted
Limed - Compacted

Figure 5