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1 **Application of X-ray tomography to evaluate liming impact on earthworm burrowing**
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1. Introduction

In the 1980s, important forest decline was observed in numerous sensitive regions throughout the Northern hemisphere due to acidic atmospheric depositions caused by human activities (Campbell and Lee, 1996; Driscoll et al., 2001). Acidic atmospheric deposition can decrease forest soil pH, indirectly induce earthworm abundance, and allow their replacement by enchytraeids in acidic soils (Edwards and Bohlen 1996; Graefe and Beylich, 2003; Rätty and Huhta, 2004). To counteract increasing soil acidity and forest dieback, many European countries conducted large-scale liming (Hüttl and Zöttl, 1993; Formanek and Vranova, 2002; Godbold, 2003). Several studies showed an increase of earthworm populations following forest liming (Kreutzer, 1995; Theenhaus and Schaefer, 1995; Deleporte and Tillier, 1999; Potthoff et al., 2008). Similarly, the introduction of *Lumbricus terrestris*, an epi-aneic species (Judas et al. 1997) and of the endogeic earthworm *Aporrectodea caliginosa* (Robinson et al. 1996) in limed acidic forest lead to the establishment of reproducing populations.

Despite the roles of earthworm species play in many soil processes (Lavelle et al., 2006), to our knowledge, few studies have addressed the impact of forest liming on earthworm activity (Springett and Syers, 1984; Robinson et al., 1991; Judas et al., 1997; Auclerc et al., 2011). By ingesting soil, fragmenting organic matter, mixing and ejecting casts above and below ground (i.e. casting and burrowing activities), earthworms increase nutrient cycling and microbial activity in organic and mineral soil horizons (Jones et al., 1994; Shipitalo and Le Bayon, 2004; Lavelle et al., 2006). It is, however, difficult to study earthworm behaviour directly under field conditions because of the complex interactions between abiotic and biotic factors. Therefore, repacked soil is often used under laboratory conditions to describe burrowing behaviour of individual earthworm species, or to describe interspecific interactions between earthworm species (Joschko et al., 1991; Jégou et al., 1998;

Jégou et al., 2001; Capowiez et al., 2001; Bastardie et al., 2003). X-ray tomography, as a non-destructive, accurate, and rapid method allows a global representation of the organisation of soil macroporosity (Capowiez et al., 2011). In repacked soil cores, 3-D reconstructions of burrow systems using X-ray tomography have been used to assess changes in the earthworm burrowing behaviour under several conditions, including metal pollution (Nahmani et al., 2005), pesticide exposure (Dittbrenner et al., 2011), or coal fly ash fertilisation (Yunusa et al., 2009).

The present study aims to determine the impact of lime addition in an acidified forest soil on the burrowing behaviour of earthworm, by using X-ray tomography. Lime addition is an increasingly used management practice in the Vosges Mountains (North-eastern, France) to counteract soil acidity and increase forest soil fertility (Landmann and Bonneau, 1995; Bonneau, 2005; van der Heijden et al., 2011). The objectives of this study include: 1) quantification of earthworm burrow structural features (mean diameter, burrow number, burrow distribution by soil depth) of the endogeic *Aporrectodea caliginosa* Savigny and the anecic *A. giardi* Ribaucourt species in limed and non-limed soils and 2) comparison of the burrowing and surface casting activities of these earthworms in recent (*in vitro*) versus prior (*in situ*) limed soils. We expect an increase of earthworm activities (burrowing and casting) by *in vitro* and *in situ* lime addition into the acidic forest soil. To this extent, we used cores with repacked soil sampled in the Vosges Mountains under laboratory conditions.

2. Material and Methods

2.1 Soils and earthworms

Soils used in this study were collected from two granitic forest catchments dominated by beech (*Fagus sylvatica*) located in the Vosges Mountains (North-eastern, France): a non-limed control catchment (47°57'39.5''N – 006°53'05.1''E; Altitude: 1060 m), and an adjacent, limed catchment (47°57'22.9''N – 006°52'55.6''E, Altitude: 1045 m). The calcareous amendment (dolomite) was spread by helicopter in October 2003 (i.e. 6 years before the laboratory study) as a fine powder composed of 70 % CaCO₃, 17 % MgCO₃, 10 % CaSO₄ and 3 % KCl at 2.5 t ha⁻¹.

Catchment soils are Entic Podzols according to WRB Classification (2006). Soil texture in the upper 30 cm of the non-limed catchment was 55.8 % sand, 22.4 % silt, and 21.8 % clay. It contained 29.3 % organic matter and had WHC of 0.16 g g⁻¹. The mean pH (measured in water) of the OH horizon was 3.8, and the A horizon was 4.5. Soil texture in the upper 30 cm of the limed catchment was 58.7 % sand, 22.9 % silt, 18.4 % clay. It contained 23.3 % organic matter and had WHC of 0.18 g g⁻¹. Mean pH in this treated catchment was 4.1 in the OH horizon and 4.7 in the A horizon.

The OH and A horizons of non-limed and limed catchments were sampled. Soils for the non-limed control came from the non-limed catchment, and soils for the *in situ* limed treatment came from the limed catchment. After field collections, OH and A horizons were sieved through 3.9 mm mesh and stored at 12°C for one week before repacking for the experiment.

The two earthworm species studied belong to two different ecological categories: *A. giardi* is anecic and *A. caliginosa* is endogeic. Adult earthworms were collected by hand-sorting from an experimental plot in La Bouzule (48°74'10.3''N – 006°32'59''E). For acclimation, earthworms were stored at 12°C for one week with 200 g of beech litter and 5 kg of non-limed OH and A horizon soil prior the start of the experiment.

2.2 Experimental procedure

The experiment was performed using PVC cylinders (15.5 cm diameter, 30 cm long) with a 1 mm nylon mesh at the bottom to allow the passage of gas and water. Three soil treatments (i.e. non-limed, *in situ* and *in vitro* limed) were established by filling the PVC cylinders with 20 cm of sieved A horizon soil, to which 5 cm of sieved OH horizon soil was added (Table 1a). For the *in vitro* limed soil treatment, we used OH horizon soil collected from the non-limed catchment into which we mixed 5 g of lime (mean pH = 5.4), which is equivalent to the 2003 catchment-level application of 2.5 t ha⁻¹. We placed this freshly limed soil above sieved A horizon soil (mean pH = 4.5) from the non-limed catchment.

Field soil bulk density (0.8 g cm⁻³) was achieved by repacking the soil and pressing it for 5 minutes with a hydraulic press at 0.75 bar. Seven layers of 0.7 kg each were sequentially added to reproduce the A horizon. Two layers of 0.4 kg each were added and packed to reproduce the OH horizon. Soil cores were acclimated in a climate chamber at 12°C ± 1°C for two days before earthworm inoculation.

Each core of three replicates per treatment was inoculated with either two individuals of *A. giardi* or two individuals of *A. caliginosa* (Table 1b). In total, 36 earthworms were applied. Individual *A. giardi* earthworms initially weighed 2.56 ± 0.3 g (mean ± SD, fresh weight) and *A. caliginosa* individuals weighed 0.73 ± 0.1 g. We prepared one earthworm-free control core for each soil treatment. To each core, after inoculation with earthworms, we added 5 g (fresh mass) of beech leaves - sampled from the litter layer of the non-limed and limed catchments – as a food source. We also added 150 mL of distilled water in each core to remoisten the soil and the litter during the experiment. All cores were kept in a climate

chamber at $12^{\circ}\text{C} \pm 1^{\circ}\text{C}$ with a light/dark cycle of 16/8h for 9 weeks from November 2009 to January 2010.

2.3 3-D reconstruction of the burrow system and topology analysis

After the 9 weeks incubation, each column was scanned at the INRA Nancy Centre using a medical X-ray tomograph (General Electrics; brightspeed exel) according to the method described in Capowiez et al. (1998). Columns were scanned horizontally at 130kV and 80 mA s^{-1} , providing 2-D images (1.25 mm thick every 1.25 mm) of the soil macroporosity. After a simple binarization of the 2-D images presenting two well separated peaks in the grey level histogram, the 3-D volume reconstruction was run by a specific algorithm (Pierret et al., 2002). The 3-D skeletons were also reconstructed by connecting the centroids of pores that overlapped between two successive images (Capowiez et al., 1998). Density parameters were analysed to obtain several characteristics: number of burrows, total burrow length, and total burrow volume. In order to evaluate the earthworm activity throughout the soil core, the cumulative burrow volume (cm^3) was summed to 25 cm depth. We use the slope of cumulative volume by depth relationship (expressed in $\text{cm}^3 \text{ cm}^{-1}$) as a measure of earthworm activity.

2.4 Surface earthworm casts, soil core pH and earthworm biomass

After column scanning, earthworm cast production (CP, expressed as mg of dry casts g^{-1} of fresh earthworm initial weight d^{-1}) was measured by collecting surface casts drying at 60°C for 3 days, and weighing. Afterward, each column was destructively harvested by layer and earthworms were hand-sorted. Earthworm survival was assessed by gently prodding

individuals using a Pasteur pipet. Earthworms were then rinsed with distilled water, gently dried with filter paper and weighed. Soil pH was measured by soil horizon at the end of the experiment using a 1:5 soil:water ratio.

2.5 Statistical analysis

Student t-tests or Welch tests (when the homogeneity of variance was not verified) were used to assess effects of soil and earthworm treatments on each burrow system characteristic, cast production, slope of the cumulative burrow-depth relationship, and final pH. Paired t-tests assessed the difference between initial and final pH for each individual depth of soil and earthworm treatment. All analyses were conducted using R software (R Development Core Team, 2008).

3. Results

3.1 Soil core pH

At the beginning of the experiment (T₀), after lime addition into the upper 5 cm of the *in vitro* limed soils (OH horizon), pH was 5.4 and was significantly greater than for the other soil treatments ($P < 0.01$; Table 2). The pH of the upper 5 cm soil of the *in situ* limed treatment was also significantly greater than in the non-limed treatment ($P = 0.03$; Table 2). These differences remained at the end of experiment (T_f) for each earthworm treatment ($P < 0.01$) and in control incubations (Table 2). In the A layer at T₀, the pH of the *in situ* limed treatment was significantly greater than in the two other treatments ($P < 0.01$). The final (T_f) pH in the A layer of the *A. giardi* treatment was similar to T₀ ($P = 0.19$) and T_f values were similar across soil treatments ($P = 0.10$). For the *A. caliginosa* treatment, the final A layer pH

in the *in situ* limed treatment was significantly smaller than at the start of incubation ($P = 0.02$), but pH increased in the *in vitro* limed treatment during the experiment ($P = 0.04$).

3.2 Final earthworm biomass and surface cast production

All earthworms were alive at the end of experiment, as indicated by physical reactions to Pasteur pipet stimulation. Biomass of both species decreased during the experiment for each soil treatment (Table 3). *A. giardi* lost significantly more weight in the non-limed treatment than in either limed treatment ($P = 0.02$ for non-limed vs. *in situ* limed and $P = 0.01$ for non-limed vs. *in vitro* limed treatment). They lost 59.2 % of initial biomass in the non-limed soil and 13.7 to 23.2 % in the limed treatments. For *A. caliginosa* the mass loss was not significantly different between the soil treatments and ranged from 12.2 % to 22.3 % (Table 3).

Cast production (CP) by *A. giardi* in the *in vitro* limed treatment was significantly higher than in the non-limed treatment ($P < 0.01$) but not different from the *in situ* limed treatment ($P = 0.30$; Table 3). Surprisingly, the endogeic worm *A. caliginosa* seemed to produce more surface casts than *A. giardi*. Anyway, no significant differences were found for *A. caliginosa* cast production between soil treatments and its production ranged from 63.6 to 109.6 mg g⁻¹ d⁻¹ (Table 3).

3.3 Burrowing patterns

3.3.1 3-D burrow system characteristics

Burrow networks occupied the entire column depth for all earthworm treatment (Fig. 1). *A. giardi* built large-volume, vertically oriented burrows, while *A. caliginosa* burrow

volumes were smaller and more horizontal. *A. giardi* burrowed a significantly higher total volume in the *in vitro* limed treatment than in the non-limed treatment ($P < 0.01$; Table 4), whereas the volume burrowed in the *in situ* treatment was not significantly different from the non-limed treatment. The same pattern was observed for the total burrow length ($P = 0.01$). No significant liming effect was found for the total burrow length or volume burrowed by *A. caliginosa* ($P > 0.05$; Table 4). Moreover, *A. giardi* burrowed a significantly higher volume in the *in vitro* limed treatment than *A. caliginosa* (Table 4; $P < 0.01$). However, *A. caliginosa* excavated 2 to 3 times longer (m g^{-1} of initial earthworm biomass; $P < 0.05$) and 2 to 5 times more burrows ($P < 0.04$) than *A. giardi*. Finally, the mean burrow length dug in the OH horizon (top 5 cm) was higher in the *in vitro* limed treatment than in either other soils for both species (Table 4; Fig.1).

3.3.2 Mean cumulative volume of burrows as a function of depth

The mean cumulative burrow volume versus depth relationship differed by soil treatment and species (Fig. 2). In the *in situ* limed and non-limed treatment, burrow volume increased linearly as a function of core depth regardless of species. In the *in vitro* limed treatment, this relationship showed two phases: first, a rapid and linear increase of burrow volume in the 0-5 cm layer (OH horizon), and second, a linear but slower excavated volume increase for 5-20 cm depth (A horizon). The relationship was significantly different between the *in vitro* limed and non-limed soil treatments for *A. giardi* in the OH horizon ($P = 0.02$), with the mean slope of 0.28 for non-limed, 0.17 for *in situ* limed, and $0.06 \text{ cm}^3 \text{ cm}^{-1}$ for *in vitro* limed soils. For *A. caliginosa*, the same difference was denoted ($P = 0.04$; mean slope: 0.61; 0.33 and $0.17 \text{ cm}^3 \text{ cm}^{-1}$ in the non-limed, *in situ* and *in vitro* limed treatments, respectively). In the A horizon, no significant difference was found for either species across

treatments (0.43; 0.30; 0.42 and 0.53; 0.66; 0.68 cm³ cm⁻¹ in the non-limed, *in situ* and *in vitro* limed treatments for *A. giardi*, *A. caliginosa*, respectively). In the OH horizon, the slope was significantly smaller for *A. giardi* than *A. caliginosa* in the *in vitro* limed treatment ($P < 0.01$).

4. Discussion

As expected, adding lime in the laboratory to acidified forest soils collected from the Vosges Mountains increased soil pH of the OH horizon from 4.0 to 5.4. During the 9 weeks of experiment, the lime mixed into the surface horizon affected deeper soil pH, especially in the presence of the endogeic earthworm *A. caliginosa*. Chan et al. (2004) found that *A. caliginosa*, *A. longa*, and *A. trapezoides* incorporated surface applied lime after 18 months in an acidic Australian pasture soil. Ingestion and deposition of casts below ground or by adherence on earthworm bodies transported the lime and incorporated it into deeper layers. In our laboratory experiment, we mixed the dolomite into the OH horizon to immediately assess its effect on the burrowing behaviour of earthworms. In the forest, dolomite is usually applied at the soil surface without any mixing. However, we expect that it leaches after dissolution and/or is incorporated by endogeic and anecic earthworm activities in deeper soil layers, inducing an increase of soil pH and of nutrient availability.

All earthworms of each species used in our experiment survived in the acidic soil for 9 weeks. As such, soil acidity at pH values as low as 3.8 were not lethal during this experiment. However, in the suboptimal experimental conditions, all individuals lost weight with *A. giardi* losing more weight than *A. caliginosa*. Experimental conditions recommended by Fründ et al (2010) were carefully followed in our study. They suggest that control earthworm weight loss

should be less than 30 % during long-term experiments if we are to draw valid conclusions from the experimental results. For both studied species, the inoculated earthworms lost between 12 and 23 % except for the *A. giardi* which lost 59 % of body mass in the non-limed soil. Thus, without the lime, soil pH will likely limit *A. giardi* activity.

Räty (2004) described *A. caliginosa* as a eurytopic species inhabiting a variety of habitats. Sparse populations of this species have been found at pH 3.6 (Bouché, 1972). This acid-tolerant species was also found in or introduced to and survived in soils with pH values from 3.9 to 7.0 (Robinson et al., 1996; Chan et al., 2004; Räty and Huhta, 2003 and 2004; Potthoff et al., 2008). *A. giardi* has been described as neutrophilic but tolerant to acidity and can live in soil with low organic matter (Bouché, 1972). In a laboratory study, Salmon and Ponge (1999) successfully introduced this species in a forest eumoder, in an oligomull and in a calcic eumull with soil pH ranging from 4.0 to 7.4. In our study, *A. giardi* appeared sensitive to a small pH change and lime addition was likely to improve the habitat conditions since the species lost less biomass in limed soil. This biomass loss may also be explained by the low food quality of beech leaves (protein, carbohydrates, lignin content; Curry and Schmidt, 2007). This concept is also highlighted by Vahder and Irmeler (2012) in two German forests where beech dominance was negatively correlated with the soil pH and endogeic earthworm biomass. The authors suggest that this pattern may be explained by the low quality and digestibility of beech litter.

The observed burrowing activity of *A. caliginosa* was typical for endogeic species: narrow, horizontal and long burrows (Jégou et al., 1998, 2001; Langmaack et al., 1999; Bastardie et al., 2002; Dittbrenner et al., 2011). In the non-limed and *in situ* limed soils, this species did not show any depth preference as the burrows were found at every depths of the core. This contrasts with Jégou et al. (1998) who found *A. caliginosa* burrowed to a maximum

depth of 13 cm but most activity was in the top 7-8 cm in a laboratory incubation with repacked cultivated soil from Brittany, France kept for 246 days. McKenzie and Dexter (1993) observed that the most complex region of the burrow network of *A. caliginosa* was located between 2.5 and 5 cm. Others have found *A. caliginosa* active in both the topsoil and subsoil (Joschko et al., 1991; Pitkänen and Nuutinen, 1997; Francis and Fraser, 1998; Langmaack et al., 1999). This shows that the burrowing behaviour of *A. caliginosa* is extremely dependent on soil characteristics and highlights the need for further earthworm burrow characterization. Furthermore, our study demonstrated that *A. caliginosa* had a depth preference for the topsoil (0-5 cm) after *in vitro* lime addition, which increased the pH in that depth from 4.0 to near 5.5.

In vitro liming stimulated *A. giardi* burrowing as evidenced by increased surface casting and increased soil excavation. These increases were most prominent in the top 5 cm. The burrow system produced by the anecic *A. giardi* was characterised by approximately 20 burrows; and thus differed from the single, permanent, nearly vertical channels described for the well-studied epi-anecic *Lumbricus terrestris*, (Jégou et al., 1998; Shipitalo and Le Bayon, 2004; Bastardie et al., 2005). In their 246 day incubation of a cultivated soil from the deep mineral horizon, Jégou et al. (1998) found that the burrow system excavated by two individuals of *A. giardi* often reached the bottom of the column and maximal activity occurred between 4 and 23 cm. In our study, *A. giardi* did not show any depth preference in the non-limed and *in situ* soil treatments, but it had increased activity and excavated longer burrows after *in vitro* liming, which induced the soil pH increase from 4.0 to near 5.5.

When comparing the burrow system of the two species regardless of soil pH, *A. caliginosa* burrows were at least twice the length of burrows by *A. giardi* when standardized by biomass. This contrasts markedly with the 246 day laboratory incubation by Jégou et al. (2001), who found in a cultivated soil, that *A. caliginosa* burrows were four times shorter than

in our study, and that *A. giardi* burrowed approximately the same length as in our study, and thus more than *A. caliginosa*. This may be due to differing soil nature and soil bulk density: 1.35 g cm⁻³ in their study versus 0.8 g cm⁻³ in our study. Perhaps, the worms were also searching more for food or favorable conditions (Jégou et al., 1999).

Finally, for both species, the volume, length, and number of burrows did not differ between *in situ* limed and non-limed soil. The burrow systems were similar even though *A. giardi* lost less biomass and produced more casts in the *in situ* limed treatment than in the non-limed soil. Thus, six years after field liming, the management practice did not improve the burrowing activities of either of the earthworm species studied here. This may be explained by the remaining acidic conditions of the *in situ* limed soil, which is consistent with another laboratory study that investigated the impact of liming on the casting activity of *Lumbricus terrestris* in soils of the same stand as the present paper (Auclerc et al., 2011). Our previous work showed that the liming needed to increase soil pH to 5.3 under laboratory conditions in order to enhance earthworm activity, which is greater than the non- and *in situ*-limed soils of the present study.

5. Conclusion

The use of X-ray computed tomography provides insights into understanding of the impacts of liming (a restoration method used in declined forests) on the burrowing activities of earthworm species which function as soil engineers. Liming affected the burrowing activity of the anecic *A. giardi* more than the endogeic *A. caliginosa* after a short term limed application (*in vitro* limed soil). In contrast, liming in the field six years prior to experiment

(*in situ* limed soil) did not positively effect earthworm activity. Owing to the importance of earthworm burrows for ecosystem functioning, their 3-D reconstruction by X-ray tomography is a useful tool for assessing and describing the effects of driving variables on earthworm activity across ecosystems.

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Figure captions

Figure 1

Reconstructions of 3-D burrows from X-ray computed tomography for representative incubations for each treatment. Color represents depth with blue burrows behind orange.

Figure 2

Mean cumulative burrow volume by soil depth for (a) *Aporrectodea giardi* and (b) *Aporrectodea caliginosa*.

Fig. 1

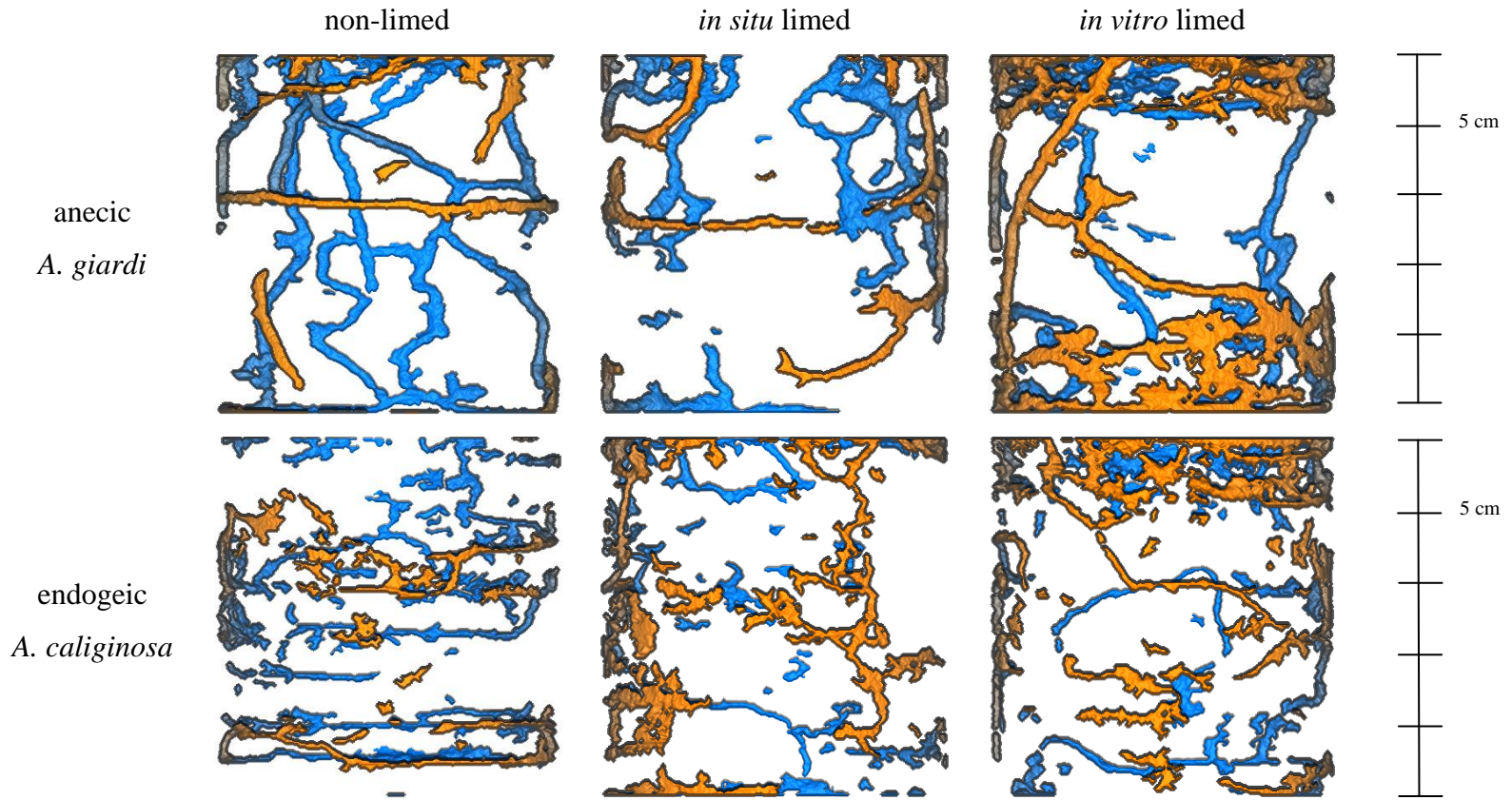
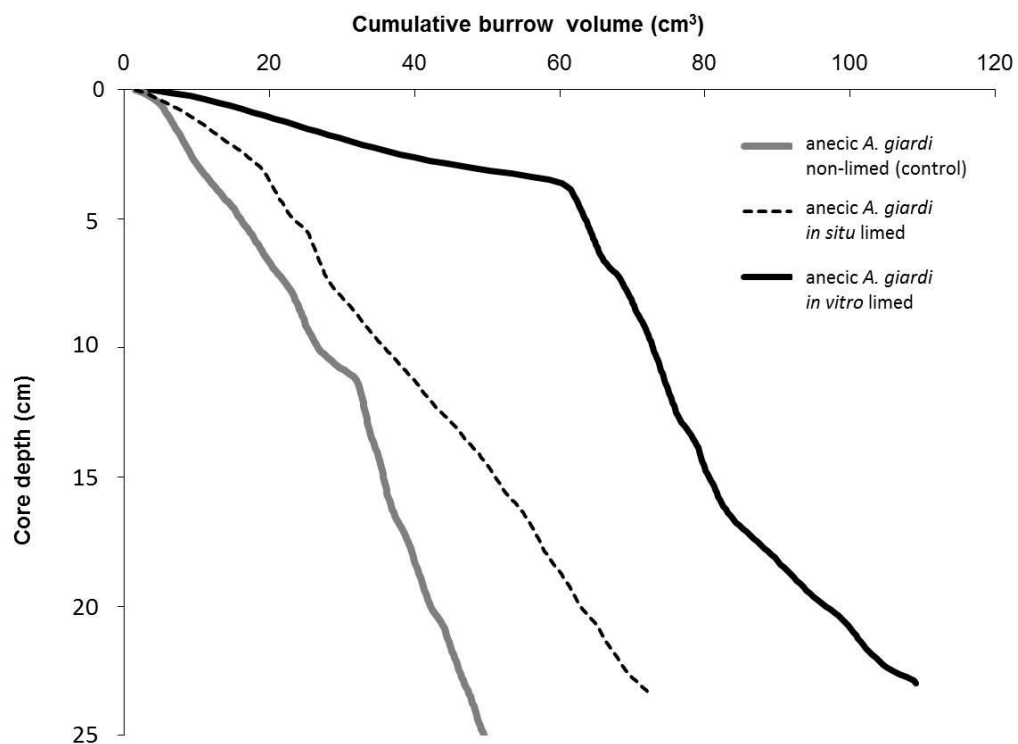


Fig. 2

(a)



(b)

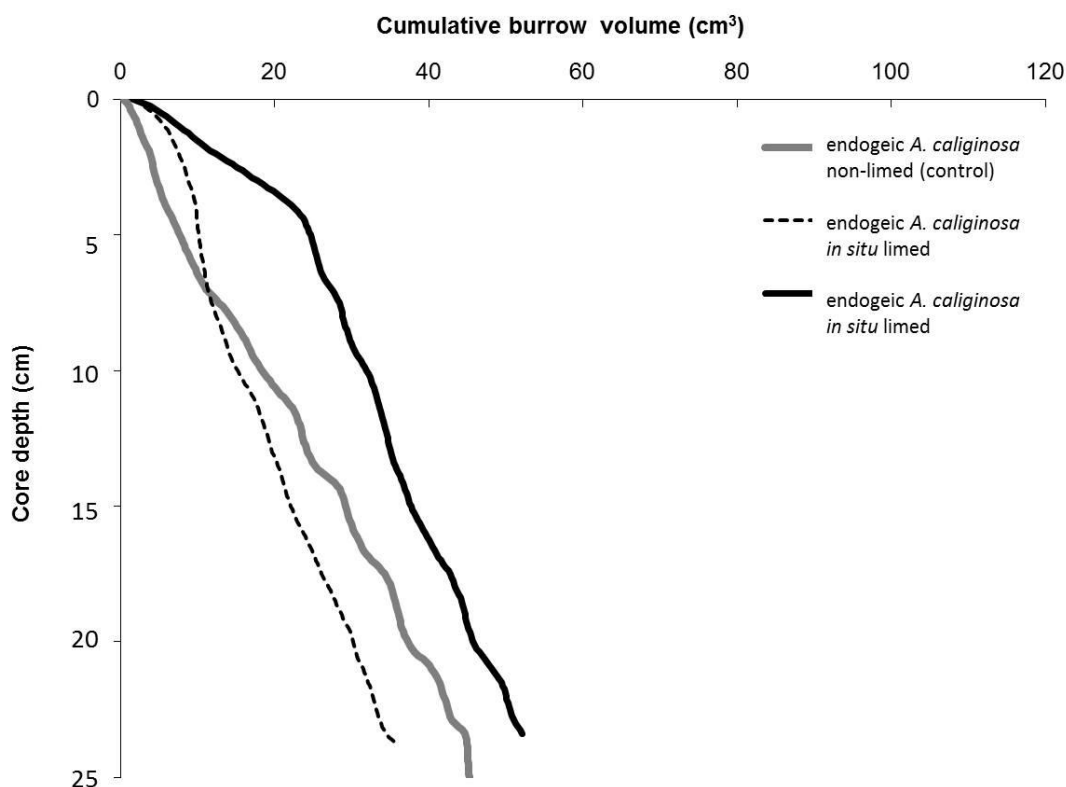


Table 1

Laboratory experiment soil source, lime addition, and earthworm treatments: (a) liming soil treatments by depth (b) total initial mass of two individual earthworms introduced to soil replicates by species.

(a)

Soil treatments	Soil in 0-5 cm	Soil in 5-25 cm
non-limed	non-limed OH horizon	non-limed A horizon
<i>in situ</i> limed	<i>in situ</i> limed OH horizon	<i>in situ</i> limed A horizon
<i>in vitro</i> limed	non-limed OH horizon + 5 g lime	non-limed A horizon

(b)

Earthworm treatments	Species	Total biomass inoculated (Mean (SD) initial fresh weight, g)
Anecic	<i>Aporrectodea giardi</i> (2 individuals)	5.27 (0.61)
Endogeic	<i>Aporrectodea caliginosa</i> (2 individuals)	1.52 (0.20)

1 **Table 2**

2

3 Initial (T0) and final (Tf) soil pH by earthworm species treatment, soil liming treatment, and depth. Soil pH values are means (SD, n=3) with
4 asterisks denoting significant differences between T0 and Tf (paired t-test) and letters denoting significant differences (P<0.05) for soil treatment
5 nested within earthworm treatment*time (t- or Welsh-tests).

6

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Species Treatments	T0			Tf			Tf			Tf		
	non- limed	<i>in situ</i> limed	<i>in vitro</i> limed	<i>Anecic A.giardi</i>			<i>Endogeic A.caliginosa</i>			Control		
Soil Treatments	non- limed	<i>in situ</i> limed	<i>in vitro</i> limed	non- limed	<i>in situ</i> limed	<i>in vitro</i> limed	non- limed	<i>in situ</i> limed	<i>in vitro</i> limed	non- limed	<i>in situ</i> limed	<i>in vitro</i> limed
OH horizon	3.8 (0.02) a	4.1 (0.1) b	5.4 (0.3) c	4.1 (0.1) * A	4.4 (0.1) B	5.4 (0.2) C	4.1 (0.1) * A	4.3 (0.1) A	5.8 (0.2) B	3.9	4.1	5.5
A horizon	4.5 (0.02) a	4.7 (0.08) b	4.5 (0.02) a	4.7 (0.2) A	4.5 (0.3) A	4.7 (0.1) A	4.5 (0.1) AB	4.3 (0.1) * A	4.8 (0.1) * B	4.3	4.3	4.7

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18 **Table 3**

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20 Final earthworm biomass (% of initial earthworm fresh biomass) and final surface casts (mg g⁻¹ day⁻¹) by species and soil treatments. Masses are
21 means (SD, n=3) with asterisk denoting significant difference (P<0.05) by species and letters denoting significantly (P<0.05) different soil
22 treatments within earthworm species (t- or Welsh-tests).

23

24

25

Species Treatment	Tf <i>Anecic A. giardi</i>			Tf Endogeic <i>A. caliginosa</i>		
	non- limed	<i>in situ</i> limed	<i>in vitro</i> limed	non- limed	<i>in situ</i> limed	<i>in vitro</i> limed
Final earthworm biomass (% initial fresh biomass)	40.8 (16.3) *a	76.8 (5.5) b	86.3 (5.3) b	84.3 (10.6) a	77.7 (9.5) a	87.8 (9.7) a
Surface cast production (mg g ⁻¹ day ⁻¹)	28.6 (21.0) a	97.5 (48.1) ab	136.7 (26.6) b	63.6 (20.2) a	109.7 (73.8) a	87.4 (26.8) a

26 **Table 4**

27 Burrow system characteristics by earthworm species and soil liming treatments. Metrics are means (SD, n=3) with asterisks denoting
28 significantly different (P<0.05) earthworm treatments and letters denoting significantly different soil liming treatments within earthworm species
29 (t- or Welsh-tests).

30

Species Treatment	Tf			Tf			31
	Anecic <i>A.giardi</i>			Endogeic <i>A.caliginosa</i>			32
	Non-limed	<i>in situ</i> limed	<i>in vitro</i> limed	Non-limed	<i>in situ</i> limed	<i>in vitro</i> limed	
Burrow Abundance (#)	12 (6.0) * a	21.7 (4.0) * a	26.7 (10.5) a	62.3 (16.0) a	51 (16.5) a	66 (4.0) a	
OH horizon Burrow Length (m)	1.7 (0.7) a	2.8 (0.5) a	10.3 (6.4) a	1.6 (0.9) a	1.9 (0.9) a	4.5 (0.4) b	
Total Burrow Length (m)	5.4 (2.0) * a	8.7 (5.3) ab	17.7 (4.3) * b	9.5 (1.4) a	6.9 (3.3) a	9.6 (2.1) a	
Total Burrow Length per g Worm (m g ⁻¹ initial earthworm biomass)	1.0 (0.3) * a	1.6 (0.9) a	3.4 (0.9) b	6.6 (2.2) a	4.3 (1.6) a	6.5 (2.1) a	
Total Burrow Volume (cm ³)	54.6 (17.7) a	75.3 (45.9) ab	116.3 (6.3) * b	46.3 (10.9) a	36.8 (18.1) a	52.9 (13.0) a	