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The interactions between soil type and earthworm species determine the properties of earthworm casts

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ABSTRACT

Earthworms are recognized to increase soil porosity, reorganize soil structure, and stimulate soil microflora and nutrient mineralization. The properties of earthworm casts should depend both on earthworm species or ecological group and on soil properties. Interactions between earthworm species and soil types have been suggested, but only poorly demonstrated. In order to better understand those interactions, two hypotheses led our study: (1) Soil type has a greater influence on cast properties than earthworm; (2) Earthworms from different species influence cast properties differently; (3) The intensity and direction of the impact of each earthworm species on cast properties vary with soil properties. Fifteen physical and chemical variables (N–NH_4^+, N–NO_3^-, total organic C and N, C/N ratio, $CaCO_3$, pH, P, K^+, Mg^{2+}, Mn^{2+}, Na^+, CEC, moisture, wettability) were measured in casts of three earthworm species (*Lumbricus terrestris*, *Allolobophora chlorotica* and *Aporrectodea rosea*) produced in three temperate soils. Univariate and multivariate analyses showed that earthworm species and soil types significantly impacted cast properties. pH, N_t, K and Mg contents were interactively altered by both factors. Multivariate analysis showed that a difference of soil type had a major impact on casts properties (62%) compared to the impact of a difference of earthworm species (10%). Cast properties were most impacted by *L. terrestris*, then by *A. chlorotica* and last by *A. rosea*. The response ratio (ratio of the properties of the casts to the properties of the bulk soil) was used to quantify the effect of earthworm species compared to the control soil. It showed a higher response of variables in casts in nutrient-rich soils, especially in casts of *L. terrestris*. The interactions between earthworm species and soil types on cast properties were discussed with regards to earthworm ecology, properties of the soil, and earthworm modifications of cast microflora.

1. Introduction

Earthworms significantly modify the physical, chemical and biological properties of the soils in which they live (Lavelle et al., 2006; Lee, 1985). They can increase soil porosity by creating burrows (Francis and Fraser, 1998; Shipitalo and Le Bayon, 2004), and they reorganize soil structure by ingesting and egesting the soil while rearranging linkages responsible for soil aggregate stability (Jouquet et al., 2008; Shipitalo and Protz, 1989). Soil passage through earthworm digestive system leads to the assimilation of organic matter by earthworms and bacteria ingested with the soil. This modifies the chemical properties of newly formed aggregates (Aira et al., 2003; Lavelle et al., 1995), i.e. earthworm cats. Cast chemical properties are also altered by changes in the microbiological activities that increase organic matter mineralization within casts, and therefore increase nutrient availability (Bityutskii et al., 2012; Chapuis-Lardy et al., 2010). These physical, chemical and microbiological modifications generally result in an increased plant growth, at least partially due to the release of mineral nutrients in earthworm casts (Chaoui et al., 2003; Scheu, 2003).

Cast properties should depend on the ingested soil material, on the life-traits of the earthworm that produced them, and probably on the interaction between both (Buck et al., 1999; Jana et al., 2010; Schrader and Zhang, 1997). Earthworm communities and activities depend on soil properties such as soil moisture, temperature, texture or soil organic matter (see Lavelle, 1988 for a review). The characteristics of the soil that they ingest are likely to be a primary factor influencing the chemical, physical and biological properties of egested casts (Jouquet et al., 2008; Lavelle, 1988; Shipitalo and Protz, 1989). Indeed, the cast content in, for example, organic matter, nitrogen or clay should depend on the corresponding soil characteristics. Additionally, casts produced by different earthworm species usually present different chemical and physical properties, especially if earthworms do not belong to the same ecological group (Zhang and Schrader, 1993; Schrader and Zhang, 1997; Aira et al., 2003). Indeed, anecic earthworms feed

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on decaying litter at the soil surface and fragments of this litter are found in their cast, while endogeic species feed on soil organic matter (Lee, 1985). Different earthworm species are also known to impact differently the communities of microorganisms found in their casts (Chapuis-Lardy et al., 2010; Decaëns et al., 1999; Lavelle et al., 1995). Taken together, soil type and earthworm species have been shown to impact cast properties but these two factors have rarely been studied together and compared.

A few studies have recently compared the properties of casts produced by several earthworm species in one soil (Bottinelli et al., 2010; Jouquet et al., 2008). Other studies compared the impact of mulch type on the properties of casts produced by one or several earthworm species (Buck et al., 2000, 1999; Flegel et al., 1998). However, experiments comparing the characteristics of casts produced by several species and in different soil types are still scarce. Schrader and Zhang (1997) used two horizons of a clay soil and a loam soil, and the two species *Lumbricus terrestris* and *Aporrectodea caliginosa.* They showed that casts had a higher organic carbon content than the bulk soil, especially in loam soil compared to clay soil, with higher values for *L. terrestris*. A larger C/N ratio was found in casts of *L. terrestris* compared to *A. caliginosa*, and the $CaCO_3$ content of casts varied with earthworm species and soil type. The authors concluded that differences in organic carbon and nutrient contents observed between species were due to a different substrate selection by earthworms and to differences in the efficiency of organic matter assimilation during the gut transit. Norgrove and Hauser (2000) also suggested that the food selectivity by earthworms might enable them to adjust their feeding regime to nutrient-poor soils. They reported a negative correlation between the nutrient and organic carbon concentrations in casts and in the surrounding soil.

It is relevant to study the interactive effect of earthworm species identity and soil properties on earthworm cast properties for three main reasons: (1) Earthworms are known to modify soil's physical and chemical characteristics at least partially through cast production (Lavelle et al., 1998; Lee, 1985); (2) Earthworm impacts on soil properties are known to influence, generally positively, plant growth (Jana et al., 2010; Scheu, 2003); (3) Earthworm casts have been found to host a high density of seeds (Decaëns et al., 2003), the germination of which is likely to be affected by cast properties. This should subsequently impact the composition and structure of plant communities (Forey et al., 2011; Willems and Huijsmans, 1994). Thus, analyzing the interaction between earthworm species and soil properties will help predict the way earthworms and soils interactively impact plant growth and seed germination (Roem et al., 2002). Jana et al. (2010) showed how *A. caliginosa* produces nutrient-rich casts in a poor soil and allows plants growing in a nutrient-poor soil to grow as well as plants in nutrient-rich soils. Doube et al. (1997) showed that the increased or reduced growth parameters of wheat, barley and faba beans in presence of *Aporrectodea trapezoides* or *A. rosea* were the result of interactions between the two earthworm species and different soil textures. However, Laossi et al. (2010a, 2010b) also showed that plant biomasses were increased by *L. terrestris* in the nutrient-rich soil only.

The objectives of our study were to evaluate through a microcosm experiment the differences between the cast physical and chemical properties of three earthworm species from two ecological groups, and to determine how these differences are affected by soil characteristics. Three hypotheses led our analysis: (1) Soil type has a greater influence on cast properties than earthworm, (2) Earthworms from different species influence cast properties differently, (3) The intensity and direction of the impact of each earthworm species on cast properties vary with soil properties.

2. Methods

2.1. Soil characteristics and preparation of microcosms

Soils were collected in February 2011 from the top layer (0–20 cm) of three different permanent grasslands in Upper-Normandy, France. Climate is temperate oceanic with a mean annual rainfall of 800 mm and a mean annual temperature of 10 °C. The soil from Saint-Adrien (N 49°22′22″, E 1°07′41″) is a rendzic Leptosol (IUSS, 2006) supporting vegetation dominated by *Brachypodium pinnatum* (L.), *Festuca lemanii* (L.) and *Carex flacca* (L.). The soil from Yvetot (N 49°36′37″, E 0°44′15″) is a NeoLuvisol-Luvisol (IUSS, 2006) supporting a vegetation dominated by *Agrostis capillaris* (L.), *Lolium perenne* (L.) and *Ranunculus acris* (L.). The soil from Yville-sur-Seine (N 49°25′11″, E 0°52′54″) is a Histosol (IUSS, 2006), where the vegetation is dominated by *Poa trivialis* (L.), *L. perenne* and *A. capillaris*. Names of soil types used here were Rendosol (Re), Luvisol (Lu) and Histosol (Hi).

All soils were hand-sieved within two days after collection with a 5 mm mesh sieve and air-dried for a week. Microcosms were cylindrical boxes (13.5 × 11 cm) filled with 750 g of soil watered with 115 mL water. When necessary, water was added to microcosms to adjust the soil water retention capacity to 65%. Microcosms were placed in the dark (17 °C) for a week to favor the establishment of naturally present microorganisms before the introduction of earthworms. Soil moisture was kept constant throughout the experiment.

2.2. Earthworms and cast collection

We used the anecic *Lumbricus terrestris* (L.) and the endogeics *Allolobophora chlorotica* (Sav.) and *Aporrectodea rosea* (Sav.), which are commonly found in grassland ecosystems of North-West France (Decaëns et al., 2008). Moreover, while casts of *L. terrestris* are commonly studied, casts of *A. chlorotica* (Sav.) and *A. rosea* (Sav.) have been subject to lesser attention. *A. chlorotica* individuals (AC; 0.32 ± 0.08 g, average fresh weight without gut content) were hand-sampled in April 2011 in neutral grasslands outside the university campus of Mont-Saint-Aignan. *A. rosea* individuals (AR; 0.23 ± 0.04 g) were hand-sampled in alluvial deposits near the Seine River and *L. terrestris* individuals (LT; 5.23 ± 0.73 g) were purchased in a fishing bait store. After voiding their guts for 24 h on moist filter paper in Petri dishes, three adult individuals from a single species were added to each microcosm leading to a total of twelve treatments: 3 soil types × (3 earthworm species + control without earthworm). Each combination of treatment was replicated five times and all 60 boxes were kept in darkness at 17 °C for the length of the experiment.

We collected casts form each microcosm once to twice a week for 180 days. This frequency was decided in order to collect fresh cast material for the time of the whole experiment. Casts had to be collected manually from the entire microcosm in order to obtain sufficient cast material for analyses. The repeated disturbance of the microcosms was also applied to microcosms with no earthworm. All samples (control and casts) were taken at the same time. Casts from each microcosm were air-dried and stored until analysis except for moisture, NO_3^- and NH_4^+ where fresh material was used. Two individuals of *L. terrestris*, three individuals of *A. chlorotica* and ten individuals of *A. rosea* died during the experiment (11%). New individuals of similar weight and species were used to replace the missing earthworms.

2.3. Physical analyses

In each microcosm, we measured the moisture content of aggregates from the weight loss of fresh material (Soil: 10 g; Casts:

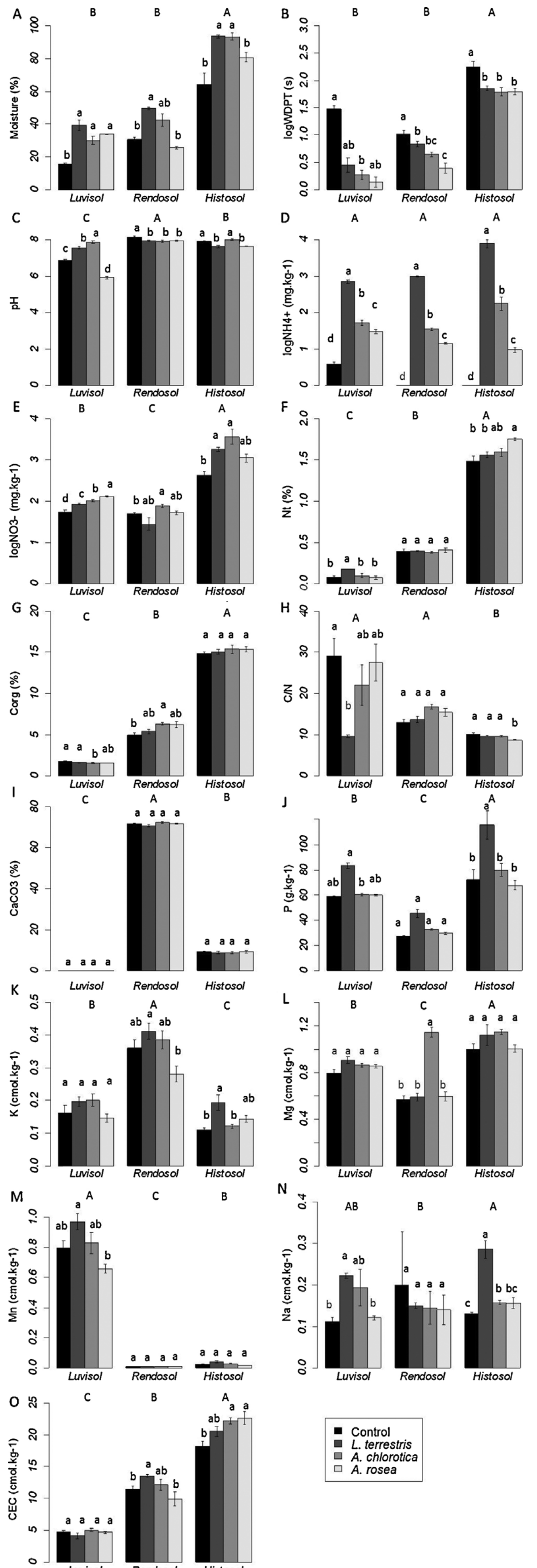

Fig. 1. Effects of earthworms and soil treatments on 15 physicochemical characteristics of soil and cast aggregates. Comparisons are within soils (lowercase letters) and between soils (uppercase letters). Similar letters are not significantly different ($\alpha = 0.05$). C: Control, LT: *Lumbricus terrestris*, AC: *Aporrectodea chlorotica*, AR: *Aporrectodea rosea*. WPDT: water drop penetration time, C_{org}, N_t: organic carbon and total nitrogen contents.

3.29 ± 0.68 g) after oven-drying at 105 °C for 24 h. The wettability of aggregates was assessed on twenty 3-to 5-mm aggregates of soil or casts by measuring water drop penetration time (WDPT) according to Chenu et al. (2000): drops of 0.1 mL de-ionized water were deposited with a micro-syringe on the surface of individually 3-to-5-mm air-dried aggregates (Hallin et al., 2011). The time elapsed between the dropping and the penetration into the aggregate was recorded.

2.4. Chemical analyses

In each microcosm, NH_4^+ and NO_3^- were extracted with a K_2SO_4 solution (0.2 M, 1:5 ratio, 1 h agitation, 100 rpm) and filtered. NH_4^+ and NO_3^- concentrations were measured by colorimetry with an AA3 auto-analyzer (BRAN + LUEBBE, Norderstedt, Germany). Total organic C and N (C_{org} and N_t) concentrations were measured with a CHN pyrolysis micro-analyzer at the IRCOF laboratory (Rouen, France) (NF ISO 10694 and NF ISO 13878, ThermoScientific, France). $CaCO_3$ content was determined by the Bernard calcimeter method. Easily assimilated P_2O_5 content (P) was measured according to Olsen (1954) at the INRA "Laboratoire d'Analyses des Sols" of Arras (France). Mg^{2+}, Mn^{2+}, K^+ and Na^+ were extracted with 25 mL of a 0.05 N cobaltihexamine chloride solution (Orsini and Rémy, 1976), and concentrations were measured by flame atomic spectroscopy (ThermoScientific, Francer). Cation Exchange Capacity (CEC) was measured by spectrophotometry at the 472 nm absorbency according to Aran et al. (2008). pH was measured with combined ThermoScientific glass electrodes (1:5 ratio, ISO 10390).

2.5. Statistical analyses

Statistical analyses were achieved with the R-software (R Core Team, 2012). Normality and homoscedasticity of the data set were tested (Shapiro-Wilk and Levene, $\alpha = 0.05$) to decide whether parametric tests (in case data met both assumptions) or non-parametric tests (in case at least one of these assumptions was not met) had to be used. Parametric tests included one-way, two-way and three-way Anova analyses. Non-parametric tests included Kruskal-Wallis tests for one-way analyses and Scheirer-Hare-Ray (SHR) tests for two-way and three-way analyses. Means were compared with Pairwise Wilcoxon tests. Significance was tested with $\alpha = 0.05$.

A principal component analysis (PCA) was performed with the ADE-4 package (Dray and Dufour, 2007) with a matrix of 60 individuals (=microcosms) and 15 variables (physicochemical characteristics) to obtain general patterns of variance. A between–within classes PCA was performed to test how the soil type alone, the earthworm species alone or the interactions of both factors affected cast properties. This method allows hierarchizing factors that contribute to the multivariate variability. The between classes PCA examines differences between groups of earthworms or group of soil types. The within classes PCA examines the variability remaining after the class effect has been removed (see Dolédec and Chessel, 1989; Dray and Dufour, 2007). The statistical significance of the between classes PCA was tested using a Monte-Carlo test based on 999-permutation (ADE-4 package). The responses of cast physicochemical properties were quantified using the natural log response ratio (LnRR), with LnRR(variable) = ln(cast properties/control soil properties). LnRR < 0 implies a negative effect of earthworms on nutrient availability within each soil, whereas LnRR > 0 implies their negative effect on nutrient availability. A linear regression LnRR was calculated on the LnRR to test the influence of soil types, earthworm species and earthworm initial weight on cast properties. Means (±standard error) presented in figures were calculated using non-transformed data.

3. Results

3.1. Relative effect of soil type and earthworm species on cast properties

We found that cast properties varied with both earthworm species identity and soil type (Table 1 and Fig. 1). Out of the 15 variables considered, 13 were significantly influenced by the soil treatment. Moisture content, WDPT, NO_3^-, total nitrogen (N_t), organic carbon (C_{org}), P and CEC were the highest in Hi. N_t, C_{org} and CEC were the lowest in Lu, NO_3^- and P were the lowest in Re, and C/N was the lowest in Hi (Fig. 1A,B,E–H, and Table 1 for *p*-values). $CaCO_3$ was the highest in Re and the lowest in Lu (Fig. 1I). Mn content decreased in this order: Lu > Hi > Re (Fig. 1M). Na content was the highest in Hi and the lowest in Re (Fig. 1N). On the other hand, 7 out of the 15 considered variables were significantly influenced by the EW treatments. The moisture content was the highest in casts produced by *L. terrestris* (LT) and the lowest in the control (C; Fig. 1A). On the contrary, WDPT was the highest in the control (Fig. 1B). NH_4^+ content was significantly different between EW species with the order: LT > AC > AR > C (Fig. 1D). P was the highest in casts of *L. terrestris* casts (Fig. 1J). Na content was highest in casts of *L. terrestris* (Fig. 1N). Finally, three variables were significantly influenced by the interaction between soil type and earthworm species identity (Table 1). pH variations were the largest between all earthworm treatments in the most acidic soil (AC > LT > C > AR; Fig. 1C). In the calcareous soil Re, casts had a lower pH than the control. Although K and pH were strongly correlated, K content was the highest in Re and the lowest in Hi (Fig. 1K). Casts of *L. terrestris* had the highest K content in Re and Hi only. Mg content was the highest in Hi and the lowest in Re, and the only difference between casts was a high Mg content in casts of *A. chlorotica* in Re (Fig. 1L). No interaction was found between the casts or the control for WDPT, CEC, NH_4^+, NO_3^-, N_t, C_{org}, C/N, $CaCO_3$, P, Na, and Mn.

The multivariate Principal Component Analysis (PCA) performed on the 15 variables and 60 microcosms confirmed that the impact of soil type on cast properties was higher than the effect of earthworm (EW) species identity (Fig. 2A and B). Axis 1 explained 43.1% of the total inertia. It was strongly positively correlated to P, NO_3^-, Mg, organic carbon (C_{org}), total nitrogen (N_t), CEC, moisture and the wettability (WDPT), and negatively correlated to Mn and the C/N ratio. This axis 1 segregated Histosol samples (negative scores) from Rendosol and Luvisol samples (positive scores) and could be associated with a gradient of nutrient availability. Axis 2 explained 22.1% of the total inertia. It was strongly positively correlated to K, $CaCO_3$ and pH and negatively correlated to Mn and P. This axis segregated Luvisol samples and *L. terrestris* samples in the Histosol (negative scores), and all samples from the Rendosol (positive scores). The most calcareous soil and the richest in K was Re, and the least calcareous and the poorest in K was Lu. Mn was significantly negatively correlated with pH, P and $CaCO_3$ ($p < 0.001$). C/N was in significant opposition with moisture, NO_3^-, N_t, P and CEC ($p < 0.001$). Axis 3 explained 9.7% of the total inertia. It was strongly positively correlated to NH_4^+ and Na (positive scores) and negatively to the WDPT (not shown). This axis segregated earthworm species. *L. terrestris* always had negative scores while scores of other species varied with soil type.

In order to hierarchize EW and soil effects, a between-within class analysis was performed on the PCA. The inertia of the between soils PCA was 9.3 and represented 61.8% of the total inertia (Table 2).

Table 1
Effect of earthworms species (EW) and soil types on 15 physicochemical variables in three different soil types. Scheirer–Hare-Ray (SHR) non-parametric test and 2-ways ANOVA (for pH and K) were used. The table shows corresponding *H*- or *F*-values. df = degrees of freedom.

Treatment	df	Moisture (%)	WDPT (S)	pH[†]	NH_4^+ ($mg\,kg^{-1}$)	NO_3^- ($mg\,kg^{-1}$)	N_t (%)	C_{org} (%)	C/N	$CaCO_3$ (%)	P ($g\,kg^{-1}$)	K[†] ($cmol\,kg^{-1}$)	Mn ($cmol\,kg^{-1}$)	Mg ($cmol\,kg^{-1}$)	Na ($cmol\,kg^{-1}$)	CEC ($cmol\,kg^{-1}$)
EW	3	11.50**	10.94*	128.44***	54.27***	5.85	0.62	0.98	4.05	0.08	9.48	8.49***	1.56	13.44*	21.65***	0.74
Soil	2	38.50***	40.19***	411.85***	0.59	45.77***	52.46***	52.46***	26.5***	52.46***	42.70***	138.65***	52.20***	24.24***	7.91	52.33***
EW × soil	6	5.18	3.36	99.92***	3.04	3.76	1.65	1.83	12.59	0.55	1.00	2.52*	0.99	12.94*	2.79	1.87

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Fisher index (for pH and K).

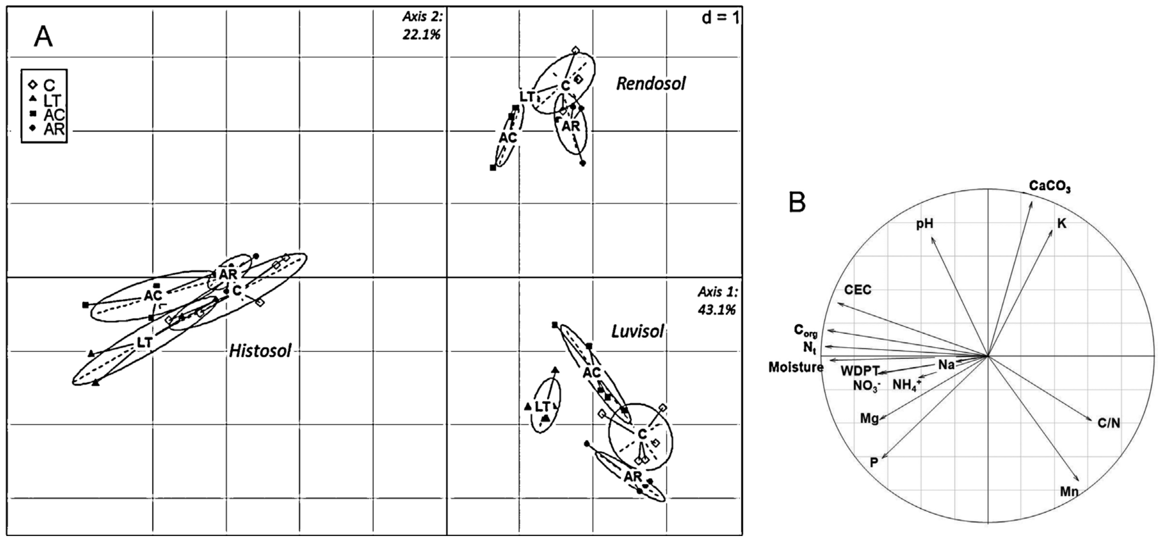

Fig. 2. Simple Principal Component Analysis (PCA) on 60 individuals and 15 physicochemical variables. (A) Projection of samples on factorial plane 1–2. (B) Correlation circle for the axes 1 and 2. C: Control, LT: *Lumbricus terrestris*, AC: *Aporrectodea chlorotica*, AR: *Aporrectodea rosea.* WPDT refers to the water drop penetration time. Ellipses indicate the center of gravity of samples with 67% of samples within the ellipse.

The correlation circle of the between soil PCA (not shown) was very close to that of the simple PCA (Fig. 2B), with axes 1 and 2 accounting for 66.4 and 33.6% of the total variance respectively. This indicated that cast properties were mainly influenced by a soil effect. This result was shown to be strongly significant by the Monte-Carlo test ($n = 9999$ permutations, $p < 0.001$). The correlation circle of the within soils PCA (Fig. 3B) showed its axis 1 was close to the axis 3 of the simple PCA (Fig. 2B). Fig. 3A shows the variability of samples from one soil to another. Axis 1 was mainly explained by differences in cast properties of *L. terrestris* in the Histosol and Luvisol

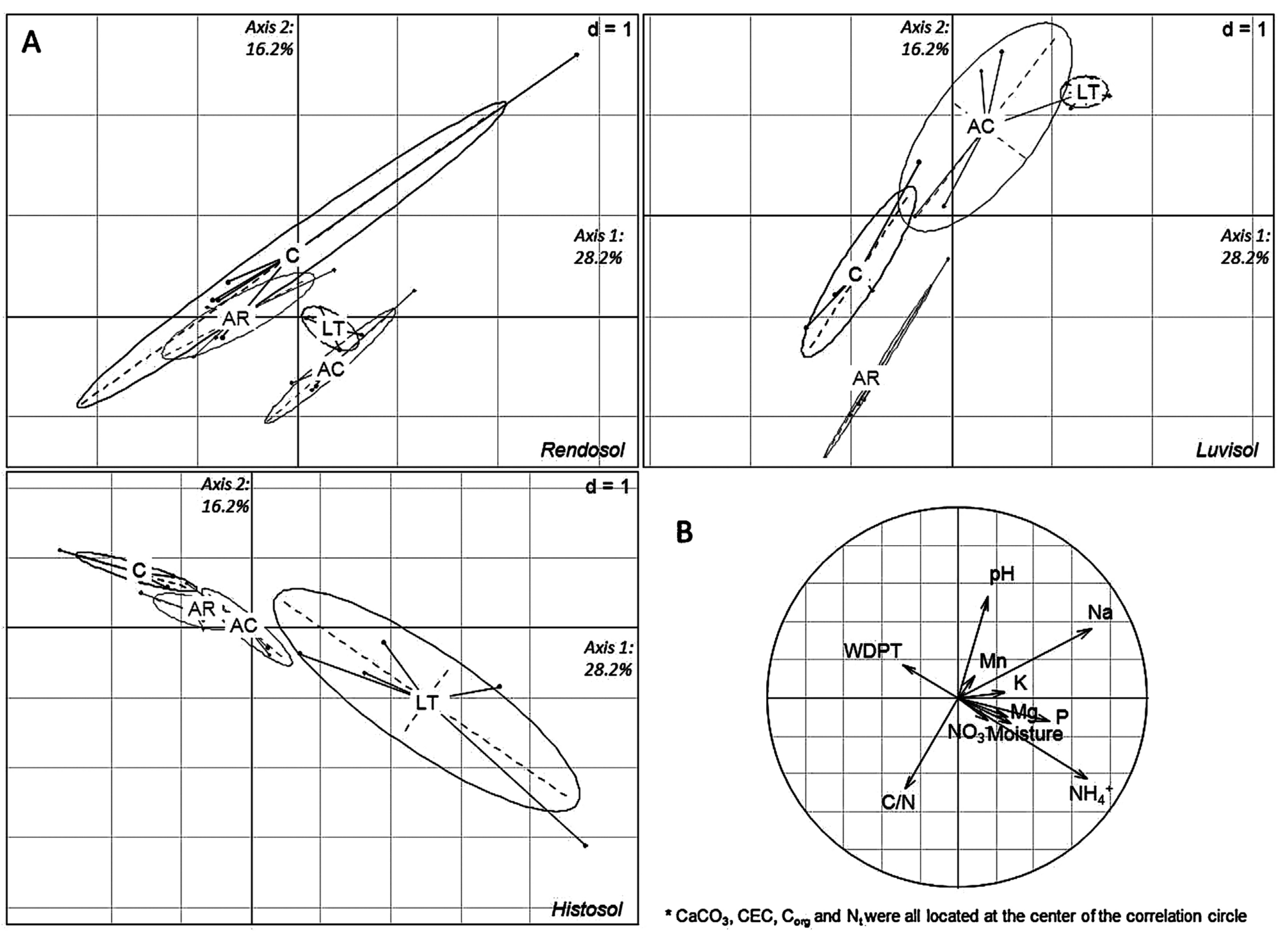

Fig. 3. Within soil Principal Component Analysis (PCA) of the 15 physicochemical variables. C: Control, LT: *Lumbricus terrestris*, AC: *Aporrectodea chlorotica*, AR:*Aporrectodea rosea.* WPDT refers to water drop penetration time. Ellipses indicate the center of gravity of samples with 67% of samples within the ellipse.

Table 2
Total inertia and first eigenvalues of the between-within class PCA analysis to test for an explanatory effect of soil type and earthworm species on the modification of cast properties. Inertia ratio indicates the percentage of variability explained by the between or within classes effect. *Residuals* indicates the percentage of the inertia that was neither explained by a soil effect nor an earthworm effect alone.

	Total inertia	First eigenvalue	Inertia ratio (%)
General PCA	15	6.95	
Between soils PCA	9.27	6.15	61.80***
Within soils PCA	5.73	1.62	38.20
Between EW PCA	1.57	0.90	10.46*
Within EW PCA	13.43	6.29	89.54
Residuals			27.74

* Significant at the 0.05 probability level, Monte-Carlo test, $n = 9999$ permutations.
*** Significant at the 0.001 probability level, Monte-Carlo test, $n = 9999$ permutations.

(higher contents of NH_4^+ and Na contents) and axis 2 was mainly explained by the casts properties of *A. chlorotica* (higher NO_3^+ content) and control soils (higher WDPT). The inertia of the between earthworms PCA was 1.6 and represented 10.5% of the total inertia (Table 2), which shows a lower effect of earthworm species on cast properties than the soil effect. This result was shown to be significant by the Monte-Carlo test ($n = 9999$ permutations, $p < 0.019$). Axis 1 of the between earthworms PCA (not shown) was close to the axis 3 of the simple PCA (Fig. 2B), except for the variables P, Mg, C/N ratio, and moisture that also contributed to axis 1 of the between earthworms PCA. The within earthworm PCA showed that 89.5% of the total inertia was not explained by an earthworm effect (Table 2). The correlation circle (not shown) was very close to the correlation circle of the sample PCA (Fig. 2B). Axes 1 and 2 accounted for 46.8 and 24.6% of the within earthworms PCA. Variables followed the axis 1 for *L. terrestris* and Control and the axis 2 for *A. chlorotica* and *A. rosea*. The remaining 27.7% that were called *residuals* (Table 2) were neither explained by an EW effect alone nor by a soil effect alone, but included interactions between both factors.

3.2. Variations in earthworm impact on cast properties with soil types

The intensity of the modifications of cast physicochemical properties varied with the earthworm species and the soil type, as demonstrated by the LnRR (Table 3). The variation of intensity was the result of an interaction between the soil type and earthworm species for the pH, C/N ratio, total N content and K and Mn contents (Table 3). Only earthworm species altered the variations of P and Mg. Only the soil altered the variations of NO_3^- content, total C content and CEC (Table 3).

L. terrestris and *A. chlorotica* significantly increased the moisture content of casts in the three soils, with a significant decrease in casts of *L. terrestris* following the gradient of soil nutrient availability Lu > Re > Hi (Fig. 4A). *A. rosea* largely increased the moisture content of its casts in the Luvisol but less in the Histosol, and decreased it in the Rendosol. The three earthworm species increased the wettability of their casts compared to the aggregates of the bulk soil (Fig. 4B). The highest wettability occurred in the Luvisol for the three earthworm species. The lowest effect on wettability was found in casts of *L. terrestris* in the Rendosol. No difference of effect was found between the Rendosol and the Histosol in casts of *A. chlorotica* and *A. rosea*. *L. terrestris* and *A. chlorotica* strongly significantly increased the cast pH in the Luvisol only (Fig. 4C). *L. terrestris* significantly equally decreased the cast pH in the Rendosol but had no effect in the Histosol. *A. rosea* strongly decreased the pH in the Luvisol, whereas the decreasing effect was lower in the Rendosol and the Histosol. All earthworms strongly significantly increased the casts NH_4^+ content (Fig. 4D). The increase was significantly higher following the gradient Lu > Re > Hi in casts of *L. terrestris* and in casts

Table 3
Intensity of the effect of earthworm species (EW), soil type and earthworm weight on 15 physicochemical variables in comparison to the bulk soil in three different soil types with the LnRR index (LnRR = Ln (Cast/Control)). Scheirer-Hare-Ray (SHR) non-parametric test and 3-ways ANOVA (for WDPT, pH, Ct, K and Mn) were used. The table shows corresponding *H*- or *F*-values. df = degrees of freedom. Underlined values: adjusted values of F after a model selection.

Treatment	df	Moisture	WDPT†	pH†	NH_4^+	NO_3^-	N_t	C_{org}	C/N	$CaCO_3$	P	K†	Mn†	Mg	Na	CEC
EW	2	6.95*	7.94**	241.33***	32.74***	2.07	3.35	0.86	3.25	0.22	29.27***	11.22***	5.42**	9.54**	7.45*	2.19
Soil	2	24.90***	85.68***	62.51***	6.82*	28.22***	4.19	25.40***	19.67***	0.30	3.65	10.70***	1.77	0.90	21.15***	13.85**
Weight	1	0.01	0.51	4.18*	0.04	0.27	1.08	0.10	0.31	0.06	0.01	0.05	1.20	1.16	0.04	0.00
EW × soil	4	5.36	1.57	156.90***	2.62	5.04	12.42*	1.82	7.85	3.29	1.01					8.80
EW × weight	2	0.03	1.18	0.08	0.01	0.28	0.97	0.37	0.04	0.29	0.01	1.09	1.31	1.44	1.89	2.73
Soil × weight	2	0.00	0.11	0.62	0.02	0.07	0.34	0.26	0.13	0.01	0.15	0.40	0.09	1.25	0.02	1.26
EW × soil × weight	4	0.13	1.36	0.59	0.09	2.49	0.36	1.01	0.72	7.34	1.75	0.69	1.32	3.59	2.97	0.70

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
Significance at the 0.1 probability level.
† Fisher index (for WDPT, pH, Ct, K and Mn).

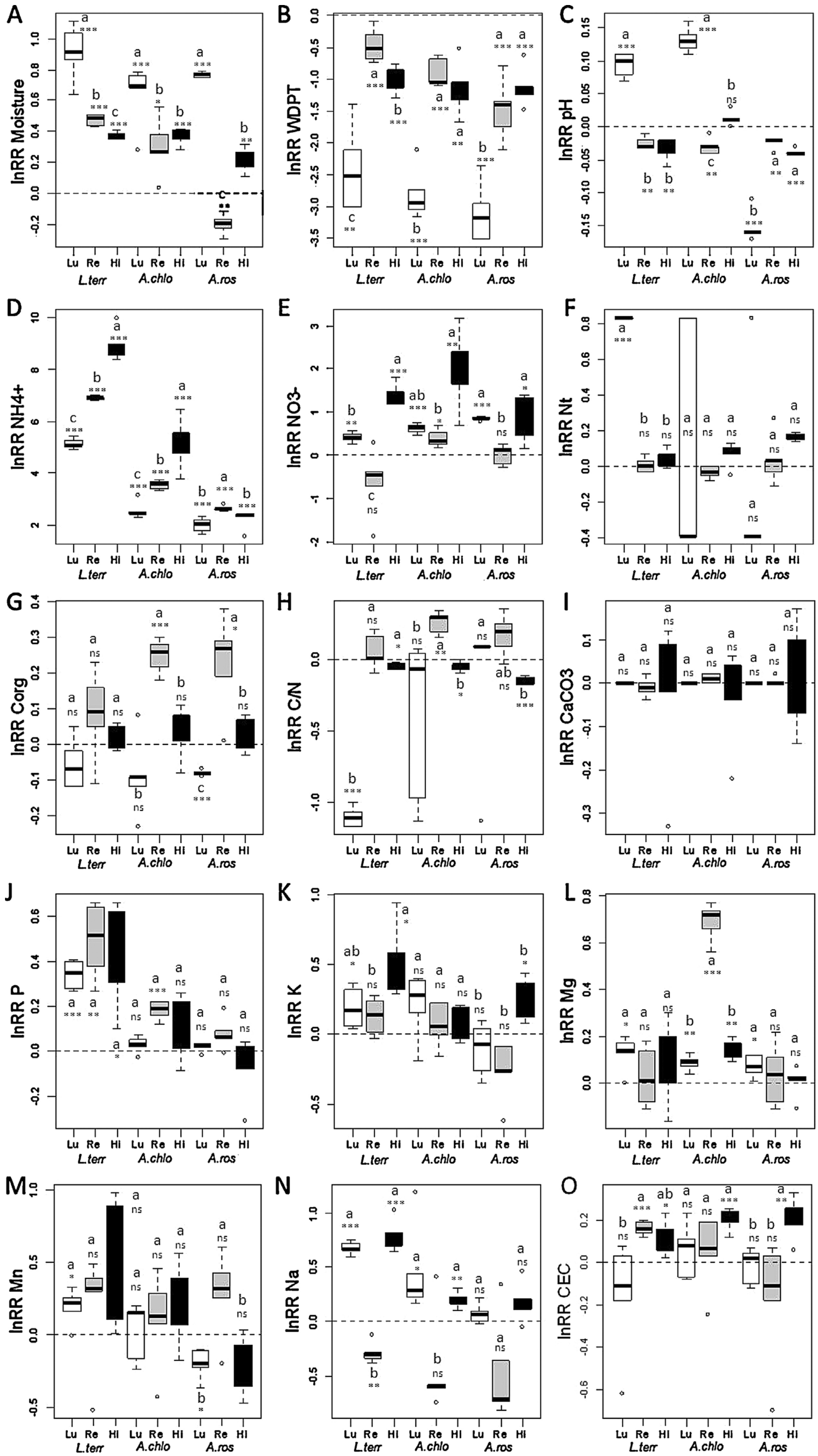

Fig. 4. Intensity of earthworm species effects (LnRR = Ln(Cast/Control)) on 15 physicochemical characteristics of cast aggregates produced by three earthworm species within three type of soils that follow a gradient of nutrient availability: Luvisol (Lu) < Rendosol (Re) < Histosol (Hi). Comparisons are within earthworm species (small letters). Similar letters are not significantly different ($\alpha = 0.05$). *Lumbricus terr*: *L. terrestris*, *Aporrectodea chlo*: *A. chlorotica*, *Aporrectodea ros*: *A. rosea*. The dotted line represents a null earthworm effect (LnRR = 0). Significant difference from 0 was tested by one-sample t-test and indicated with asterisks (ns: not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

of *A. chlorotica*. *A. rosea* had a larger effect in the Rendosol than in the two other soils. All earthworms also had an increasing effect on the content of NO_3^- in casts in all soils except *L. terrestris* and *A. rosea* in the Rendosol, where no significant effect was observed (Fig. 4E). For all earthworm species, the largest increase in NO_3^- was found in the Histosol. This effect was only found significantly different from the Luvisol in casts of *L. terrestris*. The content of N_t was only significantly increased in casts of *L. terrestris* in the Luvisol and in casts of *A. rosea* in the Histosol (Fig. 4F). Significant effects of earthworms on the organic carbon (C_{org}) were only found in casts of *A. chlorotica* and *A. rosea*, although all the effects of all earthworms followed the same pattern. *A. chlorotica* and *A.* rosea increased the C_{org} content in the Luvisol, whereas *A. rosea* decreased it in the Luvisol. No effect was found on the C_{org} content in the organic soil Histosol (Fig. 4G). Measures of C_{org} and N_t resulted in a strongly significant decrease of the C/N ratio in casts of *L. terrestris* in the Luvisol, but no significant difference in the Rendosol and Histosol. *A. chlorotica* significantly increased the C/N ratio in the Rendosol, whereas it decreased it in the Histosol and had no effect in the Luvisol. *A. rosea* only had an effect in the Histosol, where it decreased the C/N ratio significantly (Fig. 4H). No earthworm had any effect on the cast content in $CaCO_3$ within the three soils (Fig. 4I). *L. terrestris* strongly increased the P content in casts, whereas *A. rosea* had no effect. *A. chlorotica* had no effect on the P content in casts, except in the Rendosol, where it was significantly increased (Fig. 4J). *L. terrestris* significantly increased the K content in casts in the Luvisol and the Histosol only (Fig. 4K). *A. rosea* significantly increased the K content in the Histosol only. *A. chlorotica* had no significant effect on the K content in casts in any of the three soils. *A. chlorotica* strongly increased the content of Mg in casts, especially in the Rendosol (Fig. 4L). *L terrestris* and *A. rosea* only significantly increased the content of Mg in casts in the Luvisol and had no effect in the two other soils. *L. terrestris* and *A. chlorotica* significantly increased the Na content in their casts in the Luvisol and the Histosol, whereas *L. terrestris* significantly decreased the Na content in its casts the Rendosol (Fig. 4M). Although the results were not significant, *A. chlorotica* and *A. rosea* also tended to decrease the cast Na content in the Rendosol. *L. terrestris* and *A. rosea* had a significant effect on the cast Mn content in the Luvisol only (Fig. 4N). *L. terrestris* increased it, whereas *A. rosea* decreased it. Although results were not significant, *L. terrestris* and *A. chlorotica* also tended to increase the Ln content in the two other soils. *A. rosea* tended to increase the Mn content in the Rendosol but tended to decrease it in the Histosol. All the earthworm species significantly increased the CEC in casts in the Histosol (Fig. 4O). *L. terrestris* increased the CEC in the casts produced in the Rendosol, but *A. chlorotica* and *A. rosea* had no significant effect in the Luvisol or the Rendosol. Earthworm initial weight only had a significant effect on the cast pH (Table 3): a larger weight led to a higher effect on pH (Fig. 4C).

4. Discussion

4.1. Relative effect of soil type and earthworm species on cast properties

The results of our experiment show that the soil type in which earthworms live is the main explanatory factor for the modification of cast properties from the bulk soil. The soil type explained over 60% of the total variance of the PCA (Table 2), whereas the earthworm species explained only 10%. The two first axes of the PCA discriminated the three soils along a gradient of nutrient and water availability that opposed the Histosol from the Rendosol and the Luvisol. The explanation for this discrimination is that Histosols are the richest soils in carbon and organic matter, whereas Luvisols and Rendosols have much lower carbon and organic matter contents (Arrouays et al., 2001; Jones et al., 2005). In terms of water content, Luvisols and Rendosols were shown to be well drained due to a low level of clay (Batjes, 1997; IUSS, 2006), whereas Histosols are poorly drained (Batjes, 1997). When dried, Histosols increase water repellency (Dekker and Ritsema, 1996), which is also what we found in our results. Despite global tendencies, the link between soil water repellency and soil moisture, texture and organic matter is known to vary between soil types (Doerr et al., 2006). The discrimination of the Rendosol was due to the high content in $CaCO_3$ and pH that are typical of calcareous soils (IUSS, 2006).

The within-soil PCA analysis that removed the variability explained by differences between soil types showed that earthworm species impacted cast properties in contrasted ways, and that this impact varied with the soil type. We found few significant differences in organic carbon content and total nitrogen in the casts compared to the control soil. The organic carbon content only increased in casts in the Rendosol and the total nitrogen only significantly increased in casts of *L. terrestris* in the Luvisol. Most studies have found an increased organic carbon content in casts (Jouquet et al., 2008; Schrader and Zhang, 1997; Zhang and Schrader, 1993). Schrader and Zhang (1997) also showed a general higher and clearer increase of organic carbon in casts of *L. terrestris* compared to casts of *A. caliginosa* in the clayey soil. On the other hand, Basker et al. (1994) found similar contents of organic carbon in casts of *A. caliginosa* and the control in their silty-clay soil. The C/N ratio showed that only *L. terrestris* significantly increased the nitrogen decomposition in the Luvisol. No difference between earthworm species was observed in the other soils, except for an increased decomposition in casts of *A. rosea* in the Histosol.

The three earthworm species increased the cast NH_4^+ and NO_3^- in all soils, although the increase was not always significant in the Rendosol. The increase in mineral nitrogen in earthworm casts has been largely reported, especially for the ammonium content of fresh casts (Aira et al., 2003; Bityutskii et al., 2012; Decaëns et al., 1999). High levels of NH_4^+ and NO_3^- in casts are due to the ingestion of organic matter that is either directly processed by earthworms or by the earthworm microflora through a "priming effect" (Lavelle et al., 1995). Microorganisms ingested with the soil are stimulated by earthworm inner living conditions and increase the mineralization (Aira et al., 2003; Chapuis-Lardy et al., 2010; Lavelle et al., 1995). In our study, *A. chlorotica* and *A. rosea* did not significantly impact the P content, and similar results were found in casts *of A. chlorotica* (Milleret et al., 2009) and of *A. caliginosa* (Aira et al., 2003). However, a higher content of P in casts of *L. terrestris* has often been found in other studies and casts of other earthworm species (Decaëns et al., 2001; see Le Bayon and Milleret, 2009 for a review). The K content has generally been shown to increase in casts compared to bulk soils (Jouquet et al., 2008; Pommeresche et al., 2009), but it also varies with earthworm type and soil type (Basker et al., 1994). In the case of a sandy soil, epigeic *L. rubellus* increased the cast extractable K content more than *A. caliginosa*. In a silty-clay soil, K content was lower in casts than in the bulk soil (Basker et al., 1994). Our results showed no significant increase in K content between control soils and earthworm casts, except for *L. terrestris* in the Histosol. Our results also showed no significant difference in the Mg content between casts and the control within each soil, except in casts of *A. chlorotica* that were significantly enriched in Mg in the Rendosol. Other studies showed an increased Mg content in earthworm casts (Jouquet et al., 2008; Pommeresche et al., 2009), except for Basker et al. (1994), who found that both *L. rubellus* and *A. caliginosa* decreased the Mg content in the sandy soil. In our experiment, earthworms did not significantly increase the Mn content in casts compared to the control, except for *L. terrestris* that tended to increase the Mn content and *A. rosea* that decreased it. Similarly, Bityutskii et al. (2012) found a decrease of Mn content in casts of *A. caliginosa* when no litter was added. We found no significant difference in Na content between the control soil and the casts in the Rendosol, like Basker et al. (1994) in the sandy soil. The increased Na content in casts of earthworms in the Luvisol and Histosol was also observed in previous studies (Basker et al., 1994; Pommeresche et al., 2009).

We found no difference between soil and cast content in $CaCO_3$, although an increase of Ca content has already been observed (Basker et al., 1994; Decaëns et al., 2001; Pommeresche et al., 2009). This was probably due to the absence or the very high content of $CaCO_3$ in the Luvisol and Rendosol respectively that could mask any earthworm effect. Schrader and Zhang (1993) showed that casts and burrows of *A. caliginosa* had a particularly high content of $CaCO_3$, and Canti and Piearce (2003) showed that *L. terrestris* and *A. chlorotica* had calciferous glands that produced calcium carbonate. The production of $CaCO_3$ by earthworms might explain the increase of pH in the Luvisol that was less alkaline than the Rendosol and Histosol. The significant decrease in pH in casts of *A. rosea* in the Luvisol was probably due to other reactions in the gut of the earthworm species. In other soils, earthworms decreased the soil pH, which is not a common finding (Basker et al., 1994; Decaëns et al., 2001; Jouquet et al., 2008). The higher moisture content in casts was due to the fact that measurements were done on fresh casts that were still saturated with wet earthworm mucus (Lavelle and Spain, 2001).

Soil wettability (WDPT) was measured on air-dried casts. Several studies showed that the passage through the earthworm gut completely disperse soil particles that are fully reorganized. The newly formed aggregates have a particular microstructure and soil porosity is increased by 10–20% in casts of *L. terrestris* and *A. caliginosa, which* enables a better water infiltration (Larink et al., 2001). However, Bottinelli et al. (2010) found that the initial higher soil porosity of casts of the endogeic earthworm species *Metaphire posthuma* decreased when subjected to wetting and that they were unstable when moist.

In our study, no litter was added in order to better compare the influence of soil type vs. earthworm species. Although microcosm studies with no litter addition are not common (Jana et al., 2010), they enabled us to do a finer analysis of the impact of soils on the modifications of cast properties. Litter was shown significantly impact earthworm activity and cast properties, depending on earthworms' ecological group and on substrate palatability (Buck et al., 2000; Flegel et al., 1998; Flegel and Schrader, 2000; Lee, 1985). Anecic species feed partly on litter but live in the soil, whereas endogeic species feed on food of lower quality, i.e. soil organic matter (see Lavelle, 1988 for a review; Lee, 1985).

4.2. Variations in earthworm impact on cast properties with soil types

The intensity and orientation of the effect of each earthworm species on cast characteristics differed along a gradient of nutrient availability Luvisol < Rendosol « Histosol. The effect of *L. terrestris, A. chorotica* and *A. rosea* significantly increased along this gradient for the NH_4^+, and there were similar but non-significant tendencies for cast contents in P and K (Fig. 4). A large increase in NO_3^- in the nutrient-rich Histosol and a large increase of the C/N ratio in the nutrient-poor Luvisol for *L. terrestris* could also be observed. Even though our results only partially confirmed our hypothesis that the effect of *L. terrestris* increased along the gradient of nutrient availability, they were consistent with the previous findings of Laossi et al. (2010b). They showed that the main significant effects of earthworms on plant growth were due to *L. terrestris* and occurred in the nutrient-rich soil. On the other hand, several studies showed that the endogeic species *A. caliginosa* had more effect on nutrient content and plant biomass in nutrient-poor soils than nutrient-rich soils (Jana et al., 2010; Norgrove and Hauser, 2000; Laossi et al., 2010b). Our results did not follow the same pattern. The effect of *A. chlorotica* and *A. rosea* on other variables did not follow the gradient and varied with soil type. This suggests that we still miss a general theory to predict how earthworm impacts on soil properties vary with soil characteristics.

4.3. Impact of earthworm weight and cast collection

Among the factors that could have impacted the effect of earthworms on cast properties, we tested the initial earthworm weight. Results from the linear regression showed that earthworm weight only influences the pH. The frequent handling of earthworms in order to collect casts could have been a cause of stress for earthworms and could have impacted their activity (Bottinelli et al., 2010; Maltby, 1999). Although earthworms were not weighed at the end of the experiment, they showed a similar activity from the start to the end of the experiment, and only 15 out of the 135 initial earthworms used died during the experiment. To prevent an effect of the time of collection on our results, all casts were bulked prior to analysis (see Section 2.2). For these reasons, we do not think that earthworm stress had a significant impact on our results.

5. Conclusion

Our study focused on the impact of three earthworm species in three different soil types on 15 physicochemical soil and cast properties. Our results showed that (1) soil type had a major impact on cast properties, (2) different earthworm species impacted those properties differently, and (3) the intensity of these impacts varied between soil types for each earthworm species and, for some variables, these impacts follows linked to the gradient of soil nutrient availability. Although the three studied earthworm species belong to two different ecological groups, no generalization can be made on the respective impact of anecic and endogeic species. More species from the two ecological groups should be studied. Microbiological properties should also be measured to better understand the cast functioning under such interactions between soil types and earthworm species. Indeed, physicochemical and microbiological properties are closely interlinked and contribute together to nutrient cycling in casts (Brown et al., 2000; Scheu, 1987; Sheehan et al., 2008). On a larger scale, the impact of such soil-earthworm interactions on cast properties should be better describe to predict the subsequent effects on seed germination, seedling and plant growth (Ayanlaja et al., 2001; Decaëns et al., 2003; Forey et al., 2011; Scheu, 2003). A better understanding of the way earthworm effects on cast properties depend on soil type would help predict the effects of invasive earthworms on natural habitats (Hendrix and Bohlen, 2002). This could also be used in restoration ecology to predict the impact of reintroduced earthworms on soil properties and vegetation in specific soil conditions.

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