To what extent do ageing and soil properties influence Amynthas khami cast properties? Evidence from a small watershed in northern Vietnam

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Abstract

Understanding the variables explaining earthworm cast properties remains a key challenge in soil ecology. For this purpose, this study aimed to determine the relationships between the physical and chemical properties of earthworm casts and those of their surrounding soil environment. Surface earthworm casts (recently emitted or degraded) produced by the anecic earthworm *Amynthas khami* (Thai, 1984) and adjacent topsoil aggregates without traces of earthworm activity were sampled in 50 different locations covering a small watershed in northern Vietnam. We determined the organic carbon content, the spectral signatures of aggregates and the humic index of organic matter *via* mid-infrared spectroscopy and analysis of pH, hydrophobicity, wet aggregate stability and bulk density of soil aggregates.

While the physical properties of casts were not related to those of topsoil aggregates, correlations were measured between cast and topsoil aggregate chemical properties. The values of hydrophobicity, pH, bulk density and organic carbon were higher in casts than in topsoil aggregates, whereas the humic index values were lower in casts than in topsoil aggregates. No difference was measured between recent and degraded casts, with the exception of their spectral signatures. Interestingly, the effect of *A. khami* was more important in soils presenting a lower organic carbon content or higher humic index, whereas its influence on soil pH was constant (i.e., the same regardless of the pH of the topsoil). In conclusion, this study suggests a discrepancy between the impact of *A. khami* on soil physical and chemical properties. The results also show that the spatial variability of cast physical properties cannot be related with that of topsoil environment physical properties.

Keywords: Earthworm; aggregation; Vietnam; physical properties; chemical properties

1. Introduction

With respect to soil turnover, earthworms are one of the most important soil bioturbators. Earthworms ingest and egest large amounts of soil, so-called casts, which then significantly impact the physical and chemical properties of soil (Lee, 1985). The properties of earthworm casts vary among earthworm ecological categories (Clause et al., 2014; Hedde et al., 2013) and among species belonging to the same ecological category (Vos et al., 2019). However, the soil material ingested is probably the most important driver of cast properties (Van Groenigen et al., 2019). To better estimate the impact of earthworms on soil functioning, the relationships between soil and cast properties must be assessed. However, to the best of our knowledge, only two studies have shown statistical relationships between casts and soil properties. Hauser and Asawalam (1998) and Norgrove and Hauser (2000) showed positive correlations between the nutrient contents in soils and in casts. Using negative power relationships between the ratios in nutrients in cast and in soil and the soil concentration in nutrients, they also showed that earthworms can modify the amounts of nutrients in casts as a function of the nutrient contents in the soil. Conversely, other studies, not based on statistical relationships, found that cast physical properties, e.g., wet aggregate stability or soil bulk density, were independent of the properties of the soil environment (Barré et al., 2009; Thomas et al., 2008), suggesting that earthworms may act as buffer agents on soil physical properties. The abovementioned examples imply the need to better understand if and how the physical and chemical properties of the soil environment impact the properties of casts. In particular, a main gap lies in the fact that the influences of the soil environment on earthworm casts' physical and chemical properties have seldom been compared in the same study. In addition to the soil, the age of casts is another factor affecting the physical (Decaëns, 2000; Marinissen and Dexter, 1990) and chemical properties of casts (Decaëns et al., 1999; Scullion et al., 2003; Zangerlé et al., 2014). However, how the age or degradation stage of casts influences the relationship between cast and soil properties is unknown.

This study was therefore designed to assess the relationships between soil and cast properties. To do so, the chemical and physical properties of casts (recently emitted and degraded) produced by the anecic species *Amynthas khami* (Thai, 1984) were compared to those of topsoil aggregates within one small watershed in northern Vietnam. These casts provide an interesting and unique model for understanding the influence of the environment on earthworm cast properties because of their high resistance, abundance and large size (Hong et al., 2011; Jouquet et al., 2007). Our hypotheses were (i) that the chemical properties of casts were related to those of the surrounding topsoil, whereas the physical properties were not, and (ii) that the relationships were affected by the age of the casts.

2. Material and methods

2.1. Study area

Soil and earthworm casts were sampled in a small watershed (50 ha) at the M-Tropics long-term observatory located in Dong Cao village in northern Vietnam (20° 57'N, 105° 29'E). The climate in the region is subtropical humid with an annual mean temperature of 20°C and 1800 mm of rainfall. The dominant soil type is Acrisol (WRB) (Podwojewski et al., 2008), characterized by an average organic carbon content of 2.5% and a clay content of 50%. The soils are acidic (pH-H_2O ~ 5). Woodlands, secondary forests, meadows and fallows are the main land uses in the catchment. In this environment, the soil surface is often covered by giant earthworm casts produced by the anecic earthworm *Amynthas khami* (Hong et al., 2011). These casts can reach up to 20 cm in height but are often broken, probably by livestock trampling, human traffic, root growth and raindrop impact (Jouquet et al., 2009, 2008).

2.2. Sampling and laboratory analysis

Sampling took place in February 2017 during the winter season. A total of 50 points were sampled in the watershed (Fig. 1). At each location, 400 g of surface earthworm casts produced by *A. khami* were collected. Cast age was visually determined from their shape and casts were differentiated into recently emitted (humid) and degraded casts (greenish colour with cracks at the surface) (Fig. 2). Topsoil aggregates (2–12 cm depth) with no traces of earthworm activity were also sampled. In total, 150 samples were collected: (2 types of casts + 1 topsoil aggregate) × 50 locations. Soil samples were then oven-dried at 40 °C for 2 days and broken into smaller aggregates with a size between 10 and 13 mm.

Soil aggregate stability was assessed using the measured mean weight diameter (MWD) after immersion of 5 g of soil aggregates in 200 mL of distilled water (10 minutes), vertical sieving in the water through a column of sieves (5, 3.15 and 0.2 mm) and oven-drying at 40 °C (Le Guillou et al., 2012). Because of the few recent casts collected, aggregate stability was only measured for degraded casts and topsoil aggregates. The bulk density of aggregates was determined from 4 g of soil by the kerosene saturation method of McIntyre and Stirk (1954). The bulk density was measured only in 20 location points. The hydrophobicity of aggregates was assessed by measuring the water drop penetration time (WDPT) on four aggregates. It corresponded to the time required for a 13 µL drop of deionized water to penetrate the aggregate (Chenu et al., 2000). The pH was measured in 0.01 M $CaCl_2$ at a soil:solution ratio of 1 to 2.5. The soil organic carbon (OC) content was determined by potassium dichromate oxidation titration for aggregates previously crushed to a size of 2 mm. An aliquot of these samples was grounded < 200 µm and scanned with a spectrometer (FTIR 660, Agilent ex-Varian) in the mid-infrared spectral range from 4,000 to 400 cm^{-1} with a KBr separator and a silicon detector. The diffuse reflectance measurements were made at 2 cm^{-1} intervals and was converted to absorbance. The raw spectra were subjected to two different pre-processing steps. First, the spectral signature was measured from the dataset after taking the second derivative to remove baseline shifts and separate overlapping absorption. Absorbance measurements were selected each 20 cm interval in order to reduce the number of independent variables (from 1766 to 176) and bands associated with CO_2 interference (2500-2300 cm^{-1}) were removed. The data were then used for multivariate statistical analysis. Second, the humification index (HI) was calculated as a measure of soil organic matter aromaticity (Demyan et al., 2012; Margenot and Odson, 2016). The absorbance intensity at each wavenumber was normalized to the sum of the absorbance across 4,000-600 cm^{-1} and spectra were corrected for baseline shifts using a modified polynomial fitting (degree 2). Finally, a Savitzky-Golay smoothing filter was applied (filter order = 1 and filter length = 9) to remove noise from the spectra. HI was calculated as the ratio of the absorbance areas of aromatic C=C, ketone and quinone C=O, and/or amide C=O at 1660-1580 cm^{-1} to aliphatic C-H at 3010-2810 cm^{-1}. Absorbance areas were calculated using the tangential baseline method.

2.3. Statistical analyses

One-way ANOVAs were performed to assess differences in the soil physico-chemical properties of recent and degraded casts and topsoil aggregates. Prior to the ANOVAs, data were log-transformed (when requires) to achieve homogeneity of variances and normality, which were confirmed using Levene test and Shapiro-Wilk test. Post hoc Tukey's tests were performed to assess the statistical significance of differences between means. We also performed between-class analysis (BCA) and Monte Carlo permutation tests (1000 permutations) to visually summarize the information of the second-derivative mid-infrared spectra for recent and degraded casts and topsoil aggregates. Partial Mantel tests were performed to evaluate the Spearman rank correlations between the dissimilarity of spectral signatures of recent and degraded casts and topsoil aggregates while controlling the effect of geographic distance. The dissimilarity of spectral signatures was calculated by the Euclidean distance between samples. Spearman correlations were performed between physical and chemical properties. Differences were declared significant at the 0.05 probability level. All statistical calculations and plots were executed using R software (3.5.2 version) with the following packages: “car”, “ade4”, “ggplot2”, “spectacles”, “signal”, “geosphere” and “vegan”.

3. Results

3.1. Properties of casts and topsoil aggregates

Earthworm casts had, on average, higher values of OC (1.5-fold), pH (by 0.5 unit), bulk density (1.03-fold), WDPT (14.2-fold), and MWD (1.1-fold) and lower values of HI (1.5-fold) than did the topsoil aggregates (Table 1). Recent and degraded casts had similar values of OC, pH, bulk density, WDPT and HI. The BCA carried out on the mid-infrared spectra of soil aggregates clearly separated casts and topsoil aggregates on the first axis, which explained 64% of the total variability (Fig. 3). Recent and degraded casts were differentiated on the second axis, which represented 36% of the variability.

Bulk density was correlated with pH ($r = 0.43$, $p < 0.01$). MWD was correlated with OC ($r = 0.56$, $p < 0.001$), pH (0.38, $p < 0.05$) and HI ($r = -0.41$, $p < 0.05$). WDPT was correlated with OC ($r = 0.80$, $p < 0.001$) and HI ($r = -0.45$, $p < 0.01$).

3.2. Correlations between cast and topsoil aggregate properties

The chemical properties (OC, HI and pH) of recent and degraded casts were positively and linearly related to the topsoil aggregate properties (Fig. 4). The correlation coefficients were the highest for OC and pH and the lowest for HI. The cast:topsoil ratios of OC and HI were negatively related by a logarithmic function to the corresponding values of topsoil aggregates. Conversely, no relationship was found for pH between the cast:topsoil ratio and topsoil aggregate value. None of the physical properties (WDPT, MWD or bulk density) of casts were related to those of topsoil aggregates. However, negative relationships were measured for WDPT, MWD and bulk density between the cast:topsoil ratio and topsoil aggregate value (logarithmic functions for WDPT and MWD vs. a linear function for the bulk density). Partial Mantel tests showed a correlation between the dissimilarity of spectral signatures of degraded casts and that of spectral signatures of topsoil, while no correlation was found with recent casts (Fig. 5). The slope of the regression line (< 1) indicated that the variability between two spectral signatures of degraded casts was lower than that between two spectral signatures of topsoil aggregates.

4. Discussion

4.1. Cast *vs.* topsoil chemical properties

A. khami is an anecic species that ingests fresh organic matter and mixes it with soil (Hong et al., 2011). This feeding strategy explains the increase in soil organic carbon content and pH (1.5-fold and 0.5 unit, respectively), and the lower degree of aromaticity (1.5-fold) in the casts than in the topsoil aggregates. Similar changes have been reported in the recent meta-analysis conducted on earthworm cast properties (Van Groenigen et al., 2019). Therefore, casts can be seen as nutrient patches in the soil, and *A. khami* can be seen as heterogeneity drivers. Moreover, no difference could be observed between recent and degraded casts, confirming the high stability of *A. khami* casts to degradation by rain (Jouquet et al., 2012, 2011), as well as the persistence of these patches. At the scale of the watershed, the chemical properties of casts and topsoil aggregates were highly variable. The relationships between the chemical properties of casts and those of the topsoil aggregates confirmed that the soil properties largely controlled the chemical properties of casts (Hauser and Asawalam, 1998; Norgrove and Hauser, 2000). However, the negative relationships between the cast:topsoil ratio and the surrounding topsoil aggregate values for organic carbon and humic index suggest that the effect of *A. khami* was more important in soils presenting less organic carbon content and where the organic matter had a higher aromaticity. These results are in line with Hauser and Asawalam (1998) and Norgrove and Hauser (2000), who showed that the lower the soil organic carbon content is, the more anecic earthworm species feed on litter and incorporate organic matter into their casts.

The greater organic matter content in casts than in topsoil aggregates can partially explain the higher pH of casts than topsoil aggregates. Indeed, the mineralization of organic matter and the production of NH_4^+ during gut transit and afterwards in the soil are known to increase alkalinity (Basker et al., 1994). Another explanation could be that earthworms feed on plant materials enriched in oxalic acid and/or oxalate minerals, which are thereafter metabolized by oxalotrophic bacteria, leading to increased soil alkalinity (Martin et al., 2012). Finally, a last possibility could be that *A. khami* increased the pH of casts through the secretion of calcium carbonate by calciferous glands, as observed with Lumbricidae species (Robertson, 1936). The production of $CaCO_3$ granules is usually considered to result from an adaptation of earthworms for the regulation of blood pH and tissue fluid (Briones et al., 2008; Lambkin et al., 2011; Versteegh et al., 2014). However, more research is clearly needed to determine whether *A. khami* has this organ and whether the production of $CaCO_3$ granules is sufficient to significantly increase soil pH. Interestingly, our study also showed that *A. khami* increased pH with the same amplitude regardless of the pH of the soil (i.e., no relationship was found between the cast:topsoil ratio and the pH of the topsoil). This result was somewhat surprising and highlights the need to better understand the complex relationship between earthworm ecology and the regulation of soil pH.

4.2. Cast *vs.* topsoil physical properties

Our results confirmed previous results carried out at the same study site, showing that casts of *A. khami* have a higher bulk density, wet aggregate stability and hydrophobicity than topsoil aggregates (Jouquet et al., 2008; Bottinelli et al., 2010) and thus contribute positively to the resistance of soil to erosion (Jouquet et al., 2012). Despite the fact that wet aggregate stability and bulk density vary during cast ageing (Decaëns, 2000; Marinissen and Dexter, 1990), no difference could be measured between recent and degraded casts. Again, this lack of variation during ageing was probably due to the high resistance of casts to degradation. The wet aggregate stability of casts was at its maximum in most of the locations, while the hydrophobicity and bulk density were quite variable. Moreover, no relationship was found between the physical properties of the casts and those of the topsoil aggregates. Since physical properties were correlated with chemical properties (e.g., bulk density with pH, and hydrophobicity and wet aggregate stability with organic carbon, humic index and pH), the lack of a relationship between casts and topsoil aggregates suggests that during the formation of casts, physical changes occurred (e.g., changes to the microstructure) that directly influenced physical properties, obscuring the relationships with soil properties. Our results are therefore in line with other studies showing that the soil aggregate stability of casts (Thomas et al., 2008) and soil bulk density impacted by earthworms (Barré et al., 2009) were not directly influenced by the soil. Finally, the negative relationships between the cast:topsoil and topsoil aggregate values indicated that at high values of soil physical properties, the impact of earthworms would become null (i.e., on hydrophobicity and wet aggregate stability) or would even be reversed (i.e., on bulk density). This relationship suggests a high potential of earthworm casts to improve soil physical properties in degraded environments but a limited impact when topsoil already has a high potential to resist erosion. Because of the high risk of erosion in the humid tropics, the beneficial effects of anecic species under these conditions might be large.

4.3. Spectral signature of aggregates

While recent and degraded casts could not be differentiated by their elementary chemical and physical properties, the utilization of infrared spectroscopy allowed discrimination between casts and topsoil and between recent and degraded casts. This result confirms the relevance of infrared-reflectance spectroscopy for differentiating earthworm casts from the surrounding soil environment (Hedde et al., 2005). The results are also in line with the study of Scullion et al. (2003) and Zangerlé et al. (2014), who showed the potential of this technology for differentiating minor changes that occur during the degradation of organic matter within casts. The results from the partial Mantel tests showed a positive relationship between the spectral dissimilarity of topsoil and that of degraded casts and a lack of relationship with recently emitted casts. These results showed that the spectral variability of recently emitted casts could not be related to the spectral variability of the topsoil, suggesting important changes in the organization of soil after the consumption and excretion of casts by earthworms. They also showed that the degradation of casts reduced their spectral variability (regression slope < 1), resulting in properties that were correlated to those of the topsoil aggregates. The spectral signature measured with mid-infrared spectroscopy corresponds to soil organic and mineral composition (Parikh et al., 2014). If we assume that the mineral composition was similar between recent and degraded casts, it is likely that differences in the dissimilarity of spectral signatures of recent casts compared to those of degraded casts were due to an evolution in the quality of organic matter.

5. Conclusions

Although intertwined, cast physical and chemical properties are rarely compared while taking into account the variability that can be observed in the field or the stage of degradation of casts. Therefore, this study is the first to pinpoint a discrepancy between the impact of earthworms on soil chemical and physical properties. If recent and degraded casts conserve most of their properties, the environment appears to decisively influence the regulation of the chemical properties of earthworm casts. Since the physical properties of casts are not directly related to the topsoil environment, more studies are needed to understand the mechanisms affecting the physical changes during cast formation and how their impact affects soil functioning at larger scales (i.e., soil erosion at the watershed scale).

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Figure captions

Fig. 1. Example of earthworm casts used in this study: recently emitted cast (humid) and degraded cast (greenish colour with cracks at the surface).

Fig. 2. Map of sampling locations in the watershed of Dong Cao in northern Vietnam.

Fig. 3. Biplot showing the between-class analysis (BCA) performed on the MIR spectra of recent and degraded casts and topsoil aggregates without recent earthworm activity. Group significance was assessed by Monte Carlo tests. The most important wavenumbers contributing to the discrimination of the group samples are depicted.

Fig. 4. Organic carbon (OC) content, humic index (HI), pH, bulk density, water drop penetration time (WDPT) and mean weight diameter (MWD) plotted for recent (black circles) and degraded (grey circles) casts and for cast/topsoil ratio as a function of the topsoil aggregate value without recent earthworm activity (n= 20 for bulk density and n= 50 for other properties). Fitting regression models are presented if they are statistically significant. The black line corresponds to the line y = x or y =1.

Fig. 5. Relationship between mid-infrared spectra of casts (recent in black circles and degraded in grey circles) and topsoil aggregates. Fitting regression models are presented if they are statistically significant. The black line corresponds to the line y=x.

Table

Table 1. Organic carbon (OC) content, humic index *via* mid-infrared spectroscopy (HI), pH, bulk density, water drop penetration time (WDPT) and mean weight diameter of water-stable aggregates (MWD) in recent and degraded casts and topsoil aggregates. Data are presented as the means and standard deviations (n = 20 for bulk density and n = 50 for other properties). Values bearing different letters are significantly different.

Properties	Recent cast	Degraded cast	Topsoil	F and p-values
OC (%)	4.4 (0.7) a	4.4 (0.7) a	3.0 (0.7) b	108.1 ***
HI	15.9 (3.9) b	14.9 (3.4) b	23.3 (19.8) a	19.3 ***
pH	4.2 (0.4) a	4.2 (0.4) a	3.7 (0.2) b	78.5 ***
Bulk density (g cm^{-3})	1.40 (0.03) a	1.43 (0.06) a	1.37 (0.04) b	11.5 ***
WDPT (s)	26.1 (14.3) a	35.6 (28.8) a	2.1 (1.3) b	380.8 ***
MWD (mm)	-	7.9 (0.4) a	7.0 (0.9) b	32.1 ***

*The number of stars for the significance level indicates the p-value range (*** p < 0.001).*

Figure 1

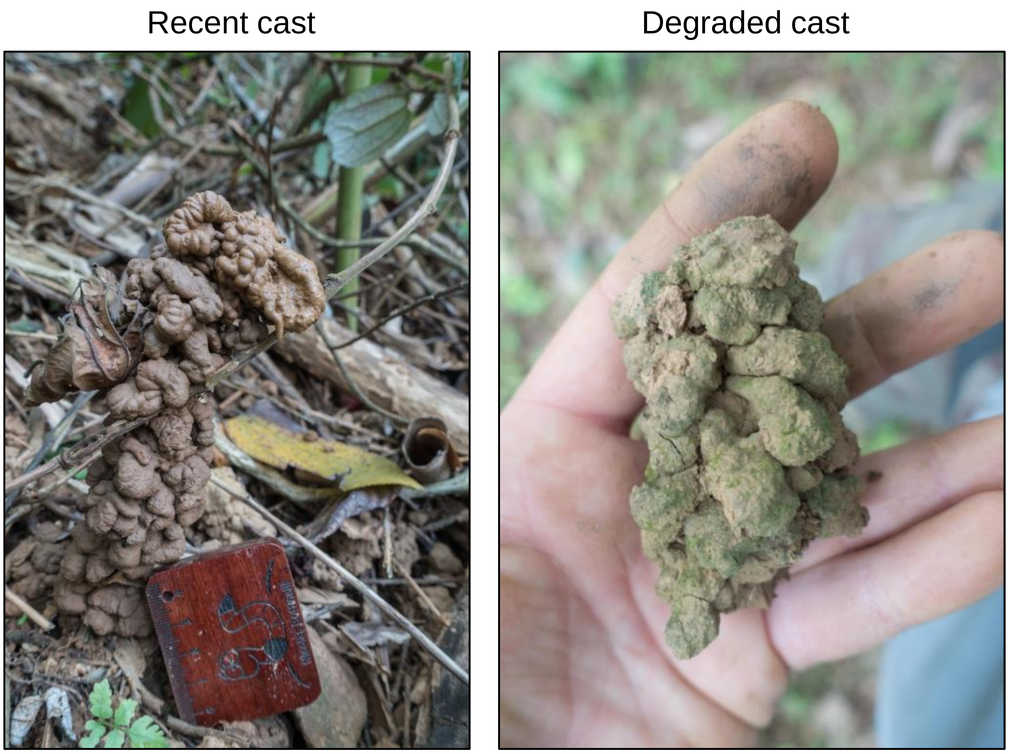


Figure 2

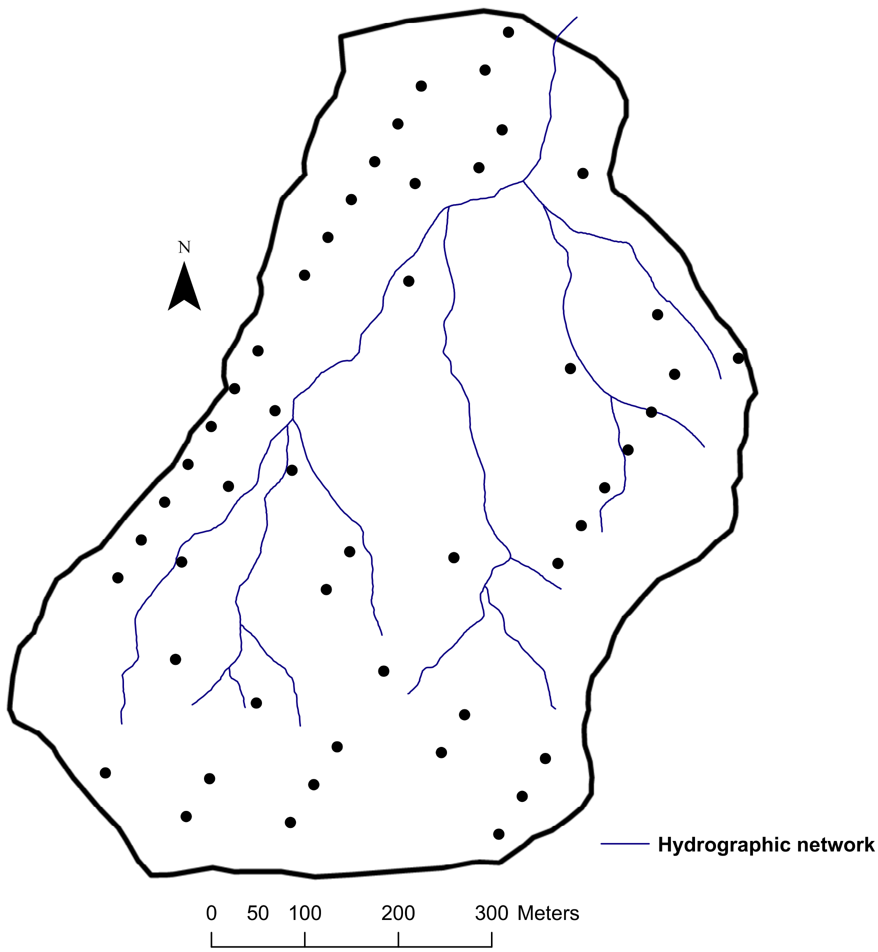
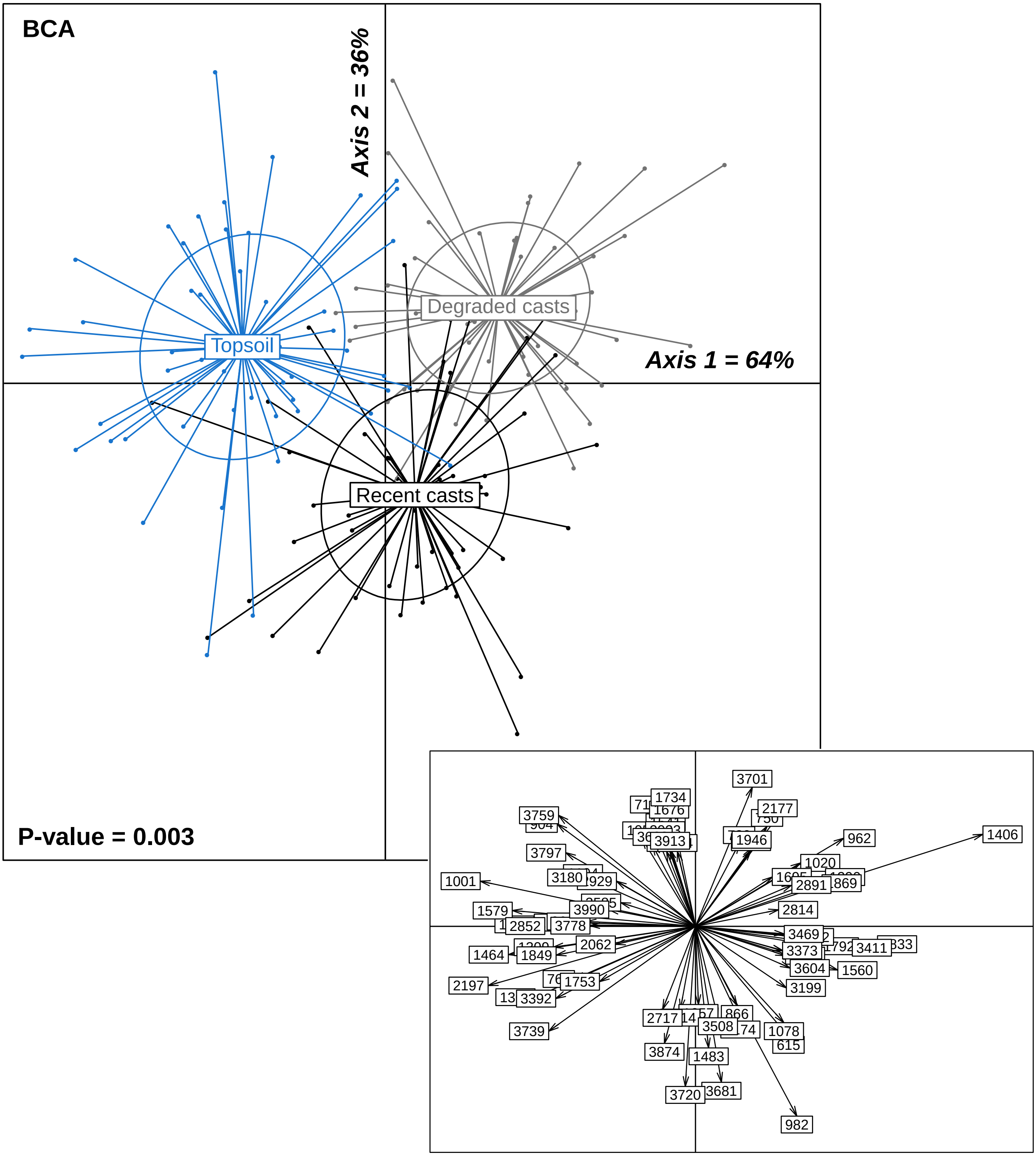
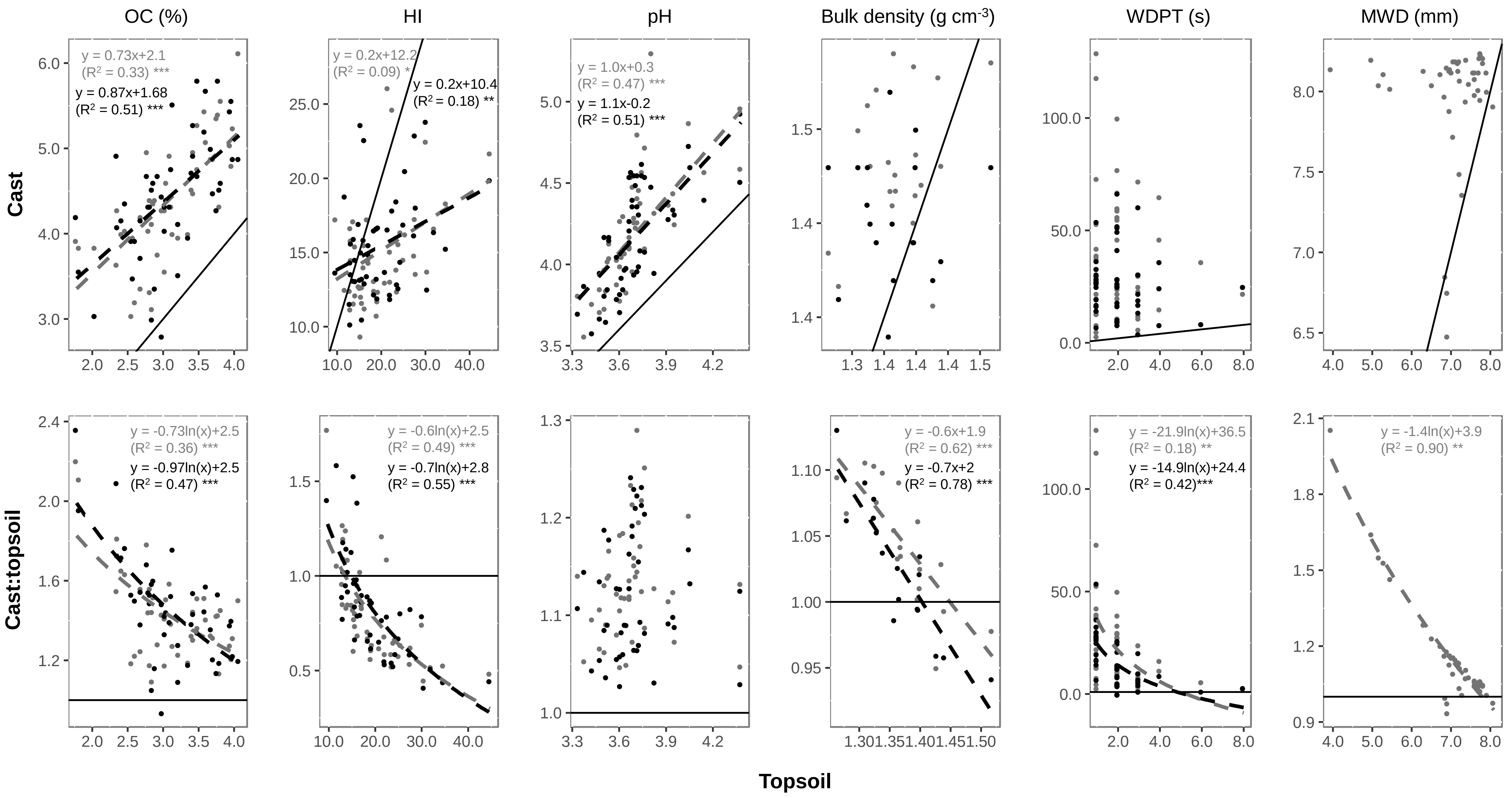
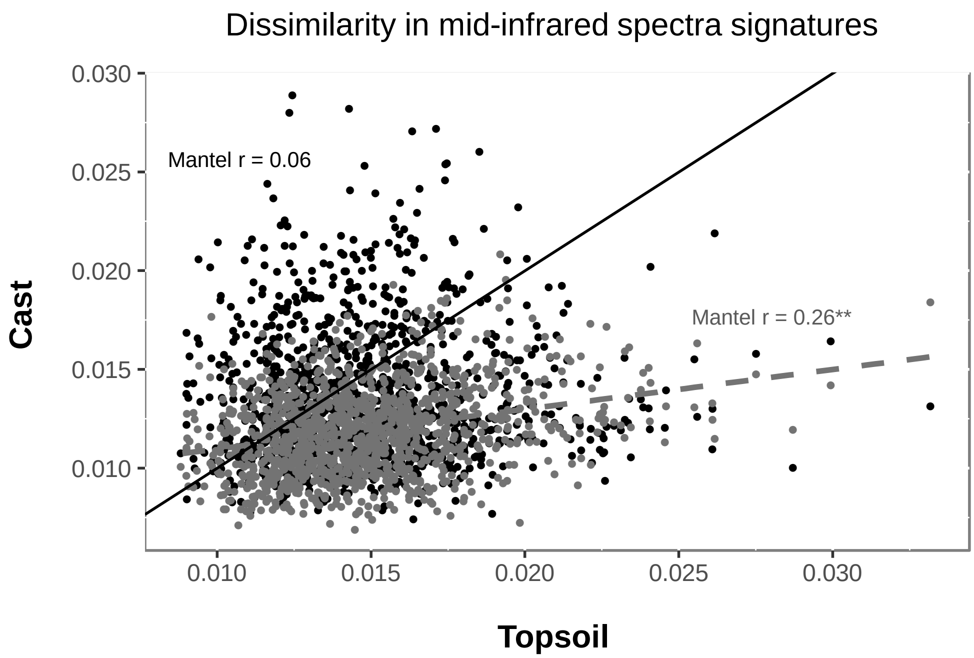


Figure 3


BCA
Axis 2 = 36%
Axis 1 = 64%
Topsoil
Degraded casts
Recent casts
P-value = 0.003
3701
1734
2177
3759
3913
1946
962
1406
3797
1020
3180
2891
869
1001
3990
2814
1579
2852
3778
3469
3373
3411
2062
3604
1560
1464
1849
3199
2197
1753
3392
866
2717
3508
1078
3739
615
3874
1483
3720
3681
982



OC (%)
HI
pH
Bulk density (g cm-3)
WDPT (s)
MWD (mm)
Cast
Cast:topsoil
Topsoil
y = 0.73x+2.1
(R2 = 0.33) ***
y = 0.87x+1.68
(R2 = 0.51) ***
y = 0.2x+12.2
(R2 = 0.09) *
y = 0.2x+10.4
(R2 = 0.18) **
y = 1.0x+0.3
(R2 = 0.47) ***
y = 1.1x-0.2
(R2 = 0.51) ***
y = -0.73ln(x)+2.5
(R2 = 0.36) ***
y = -0.97ln(x)+2.5
(R2 = 0.47) ***
y = -0.6ln(x)+2.5
(R2 = 0.49) ***
y = -0.7ln(x)+2.8
(R2 = 0.55) ***
y = -0.6x+1.9
(R2 = 0.62) ***
y = -0.7x+2
(R2 = 0.78) ***
y = -21.9ln(x)+36.5
(R2 = 0.18) **
y = -14.9ln(x)+24.4
(R2 = 0.42)***
y = -1.4ln(x)+3.9
(R2 = 0.90) **



Dissimilarity in mid-infrared spectra signatures
Mantel r = 0.06
Mantel r = 0.26**
Cast
Topsoil
0.030
0.025
0.020
0.015
0.010
0.010
0.015
0.020
0.025
0.030