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Soil chemistry changes beneath decomposing cadavers over a one-year period

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Highlights

- decomposing cadavers affect below ground soil chemistry
- cadavers cause significant increases of ammonium, nitrogen, phosphorous and potassium in the first two month
- nitrate significantly increases after eight months
- pH increased significantly at first and then decreased significantly at the end of the experiment
- chemical markers can be assigned to groups and may have the potential to date the time since death (post-mortem interval)

Abstract

Decomposing vertebrate cadavers release large, localized inputs of nutrients. These temporally limited resource patches affect nutrient cycling and soil organisms. The impact of decomposing cadavers on soil chemistry is relevant to soil biology, as a natural disturbance, and forensic science, to estimate the post-mortem interval. But cadaver impacts on soils are rarely studied, making it difficult to identify common patterns.

We investigated the effects of decomposing pig cadavers (*Sus scrofa*) on soil chemistry (pH, ammonium, nitrate, nitrogen, phosphorous, potassium and carbon) over a one-year period in a spruce-dominate forest. Four treatments were applied, each with five replicates: two treatments including pig cadavers (placed on the ground and hung one metre above ground) and two controls (bare soil and bags filled with soil placed on the ground i.e. "fake pig" treatment).

In the first two months (15-59 days after the start of the experiment), cadavers caused significant increases of ammonium, nitrogen, phosphorous and potassium ($p<0.05$) whereas nitrate significantly increased towards the end of the study (263-367 days; $p<0.05$). Soil pH increased significantly at first and then decreased significantly at the end of the experiment. After one year, some markers returned to basal levels (i.e. not significantly different from control plots), whereas others were still significantly different. Based on these response patterns and in comparison with previous studies, we define three categories of chemical markers that may have the potential to date the time since death: early peak markers (EPM), late peak markers (LPM) and late decrease markers (LDM).

The marker categories will enhance our understanding of soil processes and can be highly useful when changes in soil chemistry are related to changes in the composition of soil organism communities.

Keywords: cadaver decomposition; soil nutrients; decomposition markers; disturbance; post-mortem interval (PMI)

1. Introduction

The vast majority of decomposing organic material in terrestrial ecosystems is either plant-derived or faecal matter, while cadavers only contribute marginally (ca. 1%) (Carter et al., 2007). However, although cadaver decomposition contributes quantitatively minimally to total ecosystem nutrient cycling, it can have a locally significant, although temporally limited, impact on the soil environment (Parmenter and MacMahon, 2009). Cadavers are nutrient-rich (Barton et al., 2013) and during decomposition, they release large amounts of water and breakdown products including proteins, fats and carbohydrates, which enter the underlying soil (Dent et al., 2004)and have a major impact on soil organisms (Carter et al., 2007; Szelecz et al., 2016, 2014). Understanding these effects is relevant for both soil ecology and forensic taphonomy and may help us develop new tools for the estimation of a post-mortem interval (PMI) i.e. the time elapsed since death (Carter et al., 2010; Haglund and Sorg, 1997).

Major transitions in the decomposition process are apparent on the cadaver and lead to the division into different decomposition stages i.e. fresh, bloated, active decay, advanced decay, dry and remains (Payne, 1965). Nevertheless, decomposition is a time-continuous process with overlapping and not clear-cut stages (Goff, 2009). Various abiotic and biotic factors can influence decomposition and accordingly its impact on soils. These factors include or may include temperature (Carter and Tibbett, 2006; Carter et al., 2008), moisture (Carter et al., 2010), pH (Haslam and Tibbett, 2009), soil type (Tumer et al., 2013), season (Meyer et al., 2013), access by insects (Campobasso et al., 2001), vertebrate scavenging (DeVault et al., 2003), associated material e.g. clothing (Matuszewski et al., 2014), burial (Forbes, 2008), trauma (open wounds) (Carter and Tibbett, 2008), size, age and type of carcass (Spicka et al., 2011; Stokes et al., 2013; Towne, 2000).

A range of decomposition studies exist, differing in experimental design (e.g. cadaver types, whole bodies or only parts, buried or placed on the soil surface). These studies show effects on soil pH (Aitkenhead-Peterson et al., 2012; Benninger et al., 2008), the concentration of ammonium (Meyer et al., 2013; Stokes et al., 2009a), nitrates (Anderson et al., 2013; Meyer et al., 2013), total nitrogen (Anderson et al., 2013; Parmenter and MacMahon, 2009), total carbon (Hopkins et al., 2000; Macdonald et al., 2014), phosphorous (Macdonald et al., 2014; Towne, 2000), potassium (Aitkenhead-Peterson et al., 2012; Stokes et al., 2013), magnesium (Aitkenhead-Peterson et al., 2012) and calcium (Aitkenhead-Peterson et al., 2012; Melis et al., 2007) (Table 1 summarizes the results from the aforementioned studies that are relevant for this work). However, for some of these variables, knowledge remains very limited and the movement of carrion nutrients into soils is still an overlooked pathway (Barton et al., 2016),

We therefore investigated the impact of pig cadavers on selected soil chemical markers over a one-year period to include seasonal variation and to monitor the changes in soil chemistry beyond the peak decay stages. We compared the effects on soil chemistry of pig cadavers that were placed directly on the ground and pig cadavers that were hung one metre aboveground and contrasted them with two controls (bare soil and bags filled with soil). Our specific goals were to assess: 1) if changes in soil chemistry could be related to certain decomposition changes or time points and 2) if significant differences could be found between hanging and ground pigs.

2. Material and Methods

2.1. Study site and experimental design

The experiment was conducted in a small spruce (*Picea abies*) forest near Neuchâtel, Switzerland (47°01’05.01 N, 6°52’27.76 E, 775m a.s.l.). The study site is almost flat and covered an area of 1200 m^2. Mean temperature and total precipitation (measured in-field with a Decagon Em50 digital data logger) were 10.2 °C and 978 mm. Further details are given in Szelecz et al. (2016) (Fig. 1, p. 407). The topsoil consisted of a litter layer (spruce needles and mosses), a fragmentation layer and a humification layer (O horizon, up to 1 cm) and an umbric horizon with a dark brown colour (A horizon, 1-17 cm) (Supplementary Material Fig. S1).

In total, 20 plots (ca. 4 m distant from each other) with four treatments (five replicates each) were set up randomly: 1) control (bare soil), 2) fake pigs (cotton bags filled with soil of the same size and weight as the pig cadavers for microclimatic effects), 3) ground pigs (cadavers directly placed on the ground for microclimatic and cadaveric fluids effects), and 4) hanging pigs (cadavers hanging 1 m above ground for cadaveric fluids effects).

Ten domestic pigs (*Sus scrofa*), 8 females and 2 males, 10 weeks old, were bought from a local farm. They were sedated with Stresnil® (Azaperone) and euthanized with T61® (embutramide) by a veterinarian, immediately transported to the experimental site, weighed and placed on the plots. The average cadaver weight was 27.8 kg ± 0.8 kg (SE). All cadavers were placed in cages (140 cm x 95 cm) surrounded by wire mesh fences to keep scavengers and larger animals away. The experimental area was surrounded by an electric fence for additional protection. Control and fake pig plots were marked with bamboo sticks connected with cords. Wire mesh fences and cages could be opened at one side for soil sampling and weighing the cadavers. Cadavers were weighed just before placing and on every sampling day until D 331 using a digital hanging scale. Accordingly, soil from inside the fake pig bags was removed to match the weight loss of the pig cadavers.

2.2. Decomposition stages and sampling

Decomposition stages were estimated using the definitions provided by Payne (1965) for arthropod-exposed carrions. From the first day of cadaver placement (July, 01, 2013) until the beginning of the dry stage, each pig cadaver was examined daily to record the state of decomposition (including photographs and written reports) according to physical characteristics and arthropods present. After the beginning of the dry stage, the cadavers were examined at longer intervals (> 9 days).

On 11 sampling days from June 2013 until July 2014, a total of 220 soil samples (11 days x 4 treatments x 5 replicates) were collected. Samples were initially taken shortly before the placing of the cadavers (D0), then on days 8, 15, 22, 36, 59, 84, 123, 263, 331 and 367 (hereafter: D8, D15, D 22 asf.). A wooden rectangular frame (140 cm x 95 cm) with x (letters

A-N) and y (numbers 1-8) coordinates was placed on the ground at each site. At each sampling date, 10 points were randomly chosen from the x-y coordinates, excluding points outside of the surface directly impacted by the ground and hanging pig cadavers. These subsamples were taken with a bulb planter (6 cm diameter) to a depth of 10 cm, pooled and mixed to obtain one soil sample from each plot at each sampling day. Samples were stored at 4 °C until further processing.

2.3. Chemical analyses

Soil water pH was measured with a pH metre (Metrohm, 827 pH lab) after diluting the sample in water in a 1:2.5 proportion (Pansu and Gautheyrou, 2006). Ammonium (NH_4^+) and nitrate (NO_3^-) analyses were performed directly after sampling using colorimetric determination (Biochrom Libra S11 Spectrophotometer) (Scheiner, 2005). Total nitrogen (N) and carbon (C) were determined using a CHN analyser (Thermo Finnigan Flash EA 1112) on dry, ground soil. Bioavailable phosphorus (P_{bio}) content was determined by colorimetric analysis (Biochrom Libra S11 Spectrophotometer) according to the Olsen method (Olsen et al., 1954). Potassium (K^+) contents were determined using inductively coupled plasma optical emission spectrometry (Perkin-Elmer Optima 3300 DV ICP-OES) preceded by a cation exchange capacity extraction (CEC, cobaltihexamine method). All analyses were conducted at the Functional Ecology Laboratory, University of Neuchâtel, Switzerland.

2.4. Grouping of chemical markers

Based on the observed temporal patterns of soil chemical variables we defined three categories of markers:

(1) Early peak markers (EPM) show significantly higher concentrations in the soil beneath cadavers when compared to the controls at a certain point relatively early in the decomposition process (until the end of greatest cadaver mass loss and the end of the main leakage of cadaveric fluids).

(2) Late peak markers (LPM) show significantly higher concentrations in the soil beneath cadavers when compared to the controls at a certain point relatively late in the decomposition process i.e. not before the dry and remains stage.

(3) Late decrease markers (LDM) show significantly lower concentrations in the soil beneath cadavers when compared to the controls at a certain point relatively late in the decomposition process i.e. not before the dry and remains stage.

To be assigned to one of the categories a chemical marker had to be significantly different from both control treatments (control and fake) in at least one cadaver treatment (ground or hanging). In the case where peaks or decreases are followed by a relatively fast decrease/increase and levels discontinue being significantly higher or lower than the controls, markers are named EPM, LPM, LDM without any addition. In the case where peaks or decreases continue to be significantly higher/lower than the controls over a certain period of time either (+) EL (elevated levels) or (-) RL (reduced levels) will be added. If possible, the duration of EL or RL should be defined. Depending on their pattern, chemical markers may be attributed to one or more groups (or none if they show no pattern).

2.5. Data analyses

The duration of each decomposition stage was tested according to treatment (t-test adjusted according to Holm) to determine whether the length of the decomposition stages differed between hanging and ground pigs.

To test the significance of difference between treatments at each sampling day and overall, we used analysis of variance (ANOVA) and Tukey post hoc analysis (TukeyHSD). We assessed the significance over time using one- way ANOVA with repeated measure and post hoc multiple comparison of means (Tukey contrasts) with Bonferroni adjusted p-value. To follow the parametric assumptions of a normal distribution, variables were transformed (log 10 or square root) before the analyses.

We explored the relationships between temporal changes in soil chemical variables and treatments using redundancy analysis (RDA) on previously transformed and standardised variables. Day and treatment were used as explanatory variables and the fraction of variance explained by these variables quantified and their significance tested by Monte-Carlo permutation.

All statistical analyses were performed with R statistical software (version 3.1.0) (R Core Team, 2016) (R Core Team, 2016), and packages vegan, version 2.4.1 (Oksanen et al., 2016), nlme, version 3.1-128 (Pinheiro et al., 2016), multcomp, version 1.4-6 (Hothorn et al., 2008) and lme4, version 1.1-12 (Bates et al., 2015).

3. Results

3.1. Decomposition stages and mass loss

At the end of the experiment (D367) four of the ground cadavers and one of the hanging cadavers had reached the remains stage, while one of the ground and four of the hanging pigs were still in the dry stage (Fig. 1). The bloated stage lasted on average twice as long for the ground cadavers as for the hanging cadavers (i.e. eight vs. four days; $p < 0.05$, t-test, adjusted p-value according to Holm). However, the active decay stage was significantly longer in the hanging cadavers ($p < 0.01$, t-test, adjusted p-value according to Holm) (Fig. 1).

Cadaver mass loss followed a sigmoidal pattern with the greatest mass loss before D59. At this point all cadavers had gone through the advanced decay stage with only bones and dry skin left. The mass loss from D59 onwards was more or less constant until the end of the experiment (Fig. 2).

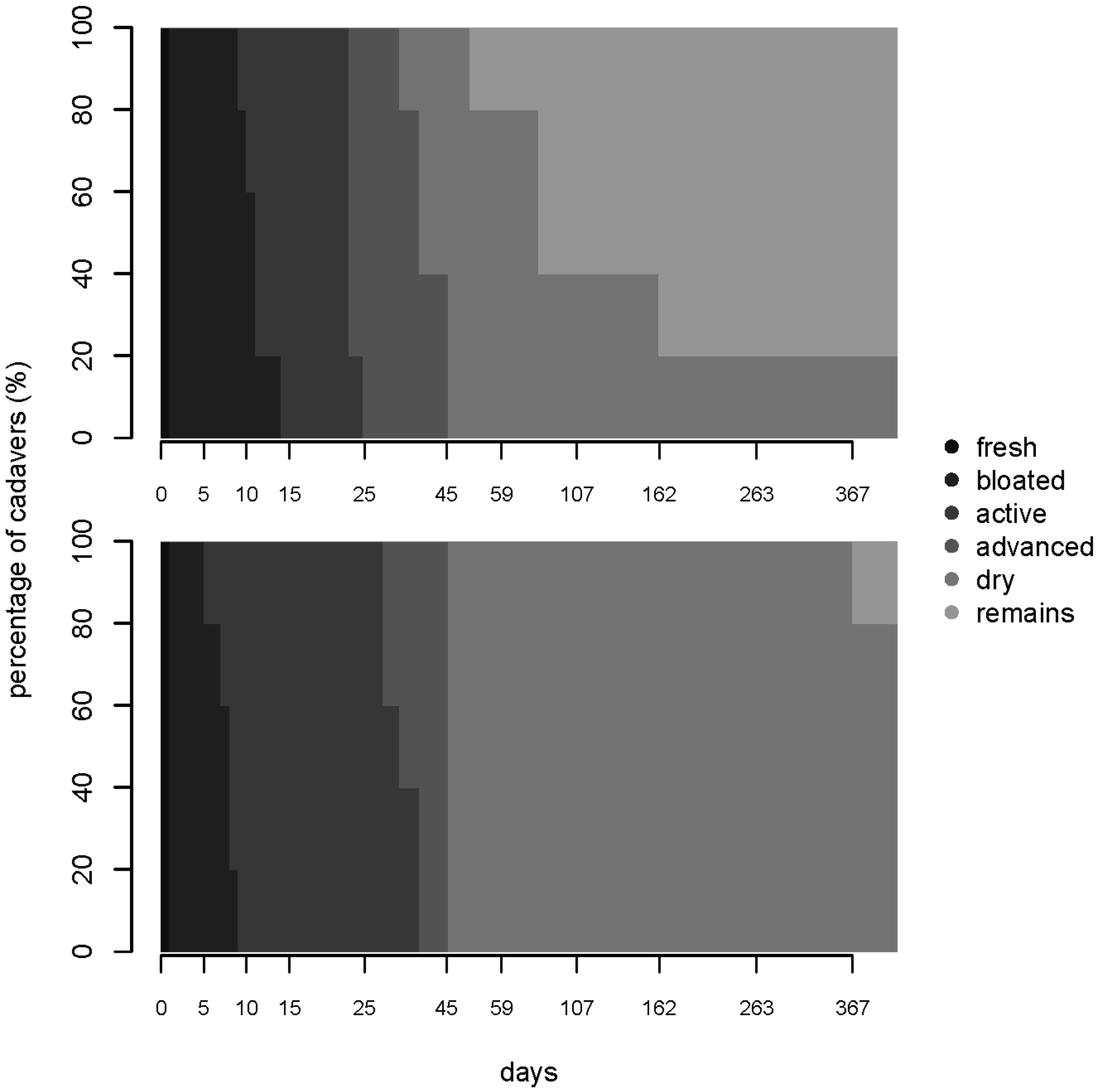

Figure 1. Duration of decomposition stages, and percentage of cadavers representing a given decomposition stage in the ground (top) and hanging pig (bottom) cadaver treatments over time at the Bois-du-Clos spruce forest experimental site (Neuchâtel, Switzerland). Decomposition stages are shown in different shades of grey.

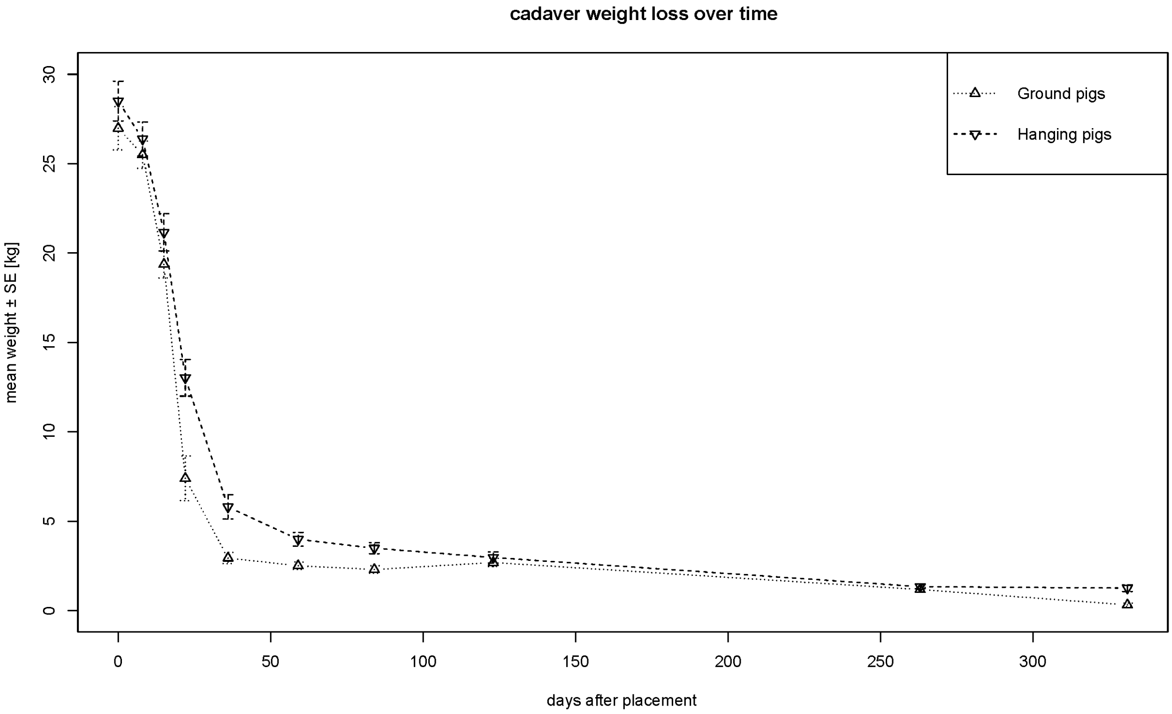

Figure 2. Average cadaver weight loss ± SE [kg] in the ground and hanging pig cadaver treatments over time at the Bois-du-Clos spruce forest experimental site (Neuchâtel, Switzerland).

3.2. Soil pH

Soil pH beneath the control and fake pigs fluctuated over the one-year period ranging from 5.05 to 7.02 (controls) and 4.71 to 6.50 (fake pigs) (Table 2). In contrast, pH beneath the ground cadavers increased by 4.13 units (ranging from 4.63 to 8.76) and was significantly different in comparison to the control and fake pig samples from days 15 to 36 ($p<0.05$, ANOVA, TukeyHSD) (Table 2, Fig. 3a). Additionally, it was significantly higher to the hanging cadavers samples on D22 ($p<0.05$, ANOVA, TukeyHSD). This increase was followed by a decrease reaching significantly lower pH values as compared to the control from D263 to D367 ($p<0.05$, ANOVA, TukeyHSD) (Fig. 3a). In comparison, the increase in pH beneath the hanging cadavers (ranging from 4.68 to 8.70) at the beginning of the experiment was weaker, but the decrease towards the end of the experiment was also significant (D263-

D367) when compared to the control and fake pig treatment ($p<0.05$, ANOVA, TukeyHSD) (Table 2, Fig. 3a).

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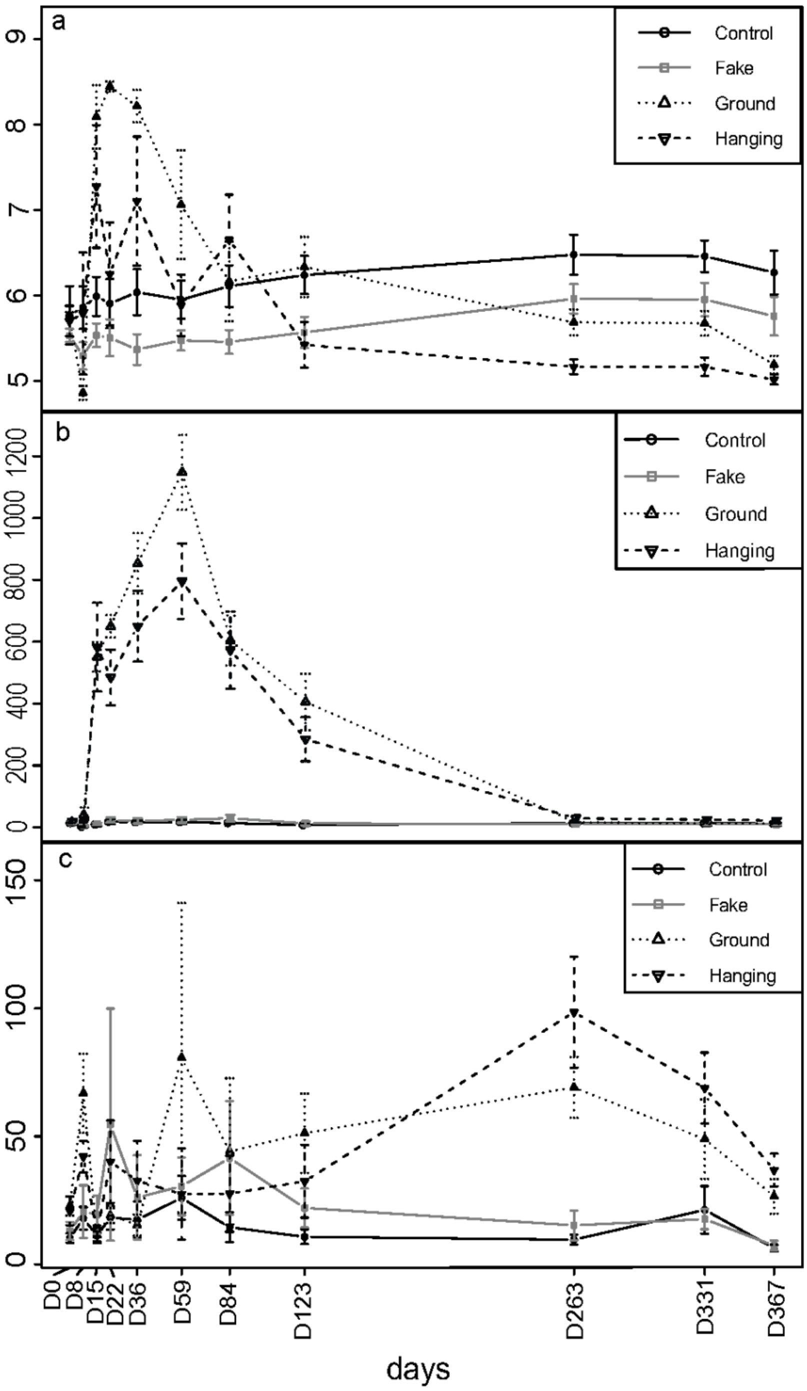
a
Control
Fake
Ground
Hanging
9
8
7
6
5
b
1200
1000
800
600
400
200
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c
150
100
50
0
D0
D8
D15
D22
D36
D59
D84
D123
D263
D331
D367
days

Figure 3. Average ± SE for pH (a), Ammonium (NH_4^+) content [µg g^{-1}] (b) and Nitrate (NO_3^-) [µg g^{-1}] (c) in the control, fake pig, ground pig and hanging pig treatments over time at the Bois-du-Clos spruce forest experimental site (Neuchâtel, Switzerland).

3.3. Ammonium (NH_4^+)

Ammonium content in the soil of the control and fake pig samples ranged from 0.92 to 50.57 µg g^{-1} in the control and 1.0 to 62.51 µg g^{-1} in the fake pig samples (Table 2). There was a massive and significant increase in Ammonium content in the ground (ranging from 1.98 to 1561.78 µg g^{-1}) and hanging pig samples (ranging from 0.64 to 1124.71 µg g^{-1}) from D15 to D123 with a peak on D59 in contrast to both controls ($p<0.0001$, ANOVA, TukeyHSD) (Table 2, Fig. 3b). Ammonium content returned to basal levels towards the end of the experiment with no significant differences between treatments on D263, D331 and D367 ($p>0.05$, ANOVA, TukeyHSD) (Fig.3b). Overall ammonium content differed significantly between cadaver treatments and controls ($p<0.0001$, ANOVA, TukeyHSD) but not between hanging and ground cadavers or between fake pigs and control ($p>0.5$, ANOVA, TukeyHSD).

3.4. Nitrate (NO_3^-)

Soil nitrate content ranged from 3.12 to 57.26 µg g^{-1} in the control samples, from 3.36 to 235.89 µg g^{-1} in the fake pig samples and from 3.7 to 321.97 µg g^{-1} in the ground and 3.67 to 164.35 µg g^{-1} in the hanging pig samples (Table 2).

Although fluctuations were observed, no significant differences were recorded between the treatments until D263 (Fig. 3c). Ground cadavers samples were significantly different from both controls on D263 and D367 ($p<0.01$, ANOVA, TukeyHSD) and hanging cadavers samples accordingly on D263, D331 and D367 ($p<0.05$, ANOVA, TukeyHSD) (Fig. 3c). Overall nitrate content differed significantly between cadaver treatments and controls ($p<0.01$, ANOVA, TukeyHSD) but not between hanging and ground cadavers or between fake pigs and control ($p>0.4$, ANOVA, TukeyHSD).

3.5. Nitrogen (N)

Total nitrogen content ranged from 0.45 to 1.95 % in the control, 0.31 to 1.55 % in the fake, 0.58 to 1.81 % in the ground and 0.57 to 2.78 % in the hanging cadavers treatment (Table 2). In the soil samples from beneath the ground and hanging cadavers nitrogen content increased at the beginning of the experiment and was significantly higher as compared to both controls on D15 and D22 ($p<0.05$, ANOVA, TukeyHSD) (Fig. 4a). Nitrogen content in the cadaver samples stayed above the controls until D331, not significantly and without any clear pattern (Fig. 4a). Overall nitrate content differed significantly between cadaver treatments and controls ($p<0.0001$), hanging and ground cadavers or between fake pigs and control ($p>0.6$, ANOVA, TukeyHSD).

3.6. Bioavailable Phosphorous (P_{bio})

Bioavailable phosphorous content in soil ranged from 4.64 to 110.86 µg g^{-1} in the control and from 0.56 to 114.41 µg g^{-1} in the fake pig samples and varied slightly over the course of the experiment. In the ground and hanging pig samples it ranged from 10.96 to 1105.3 µg g^{-1} and 13.77 to 724.42 µg g^{-1} respectively (Table 2).

In the early phase of decomposition (D15), there was a massive and significant increase in phosphorous content in both cadaver samples with a first peak on D15 and a second peak on D36 (ground cadavers) and D84 (hanging cadavers) ($p<0.0001$, ANOVA, TukeyHSD; Fig. 4b). Although phosphorous decreased again after the second peaks, the content stayed significantly higher until the end of the experiment (D367) ($p<0.01$, ANOVA, TukeyHSD; Fig. 4b). Overall phosphorous content differed significantly between cadaver treatments and controls ($p<0.0001$, ANOVA, TukeyHSD) but not between hanging and ground pigs or between fake and control ($p>0.5$, ANOVA, TukeyHSD).

3.7. Potassium (K^+) (exchangeable cation)

Potassium concentrations in soil ranged from 0 to 2.2 $cmol_c\ kg^{-1}$ in the control, 0 to 0.34 $cmol_c\ kg^{-1}$ in the fake pigs, 0 to 30.76 $cmol_c\ kg^{-1}$ in the ground cadavers and 0 to 22.93 $cmol_c\ kg^{-1}$ in the hanging cadavers treatment (Table 2). Potassium content in the control and fake pig samples did not change over the course of the experiment (Fig. 4c). However, it increased in the ground and hanging cadavers samples at the beginning of the experiment and was significantly different from both controls from D36 until D59 ($p<0.05$ ANOVA, TukeyHSD). Overall potassium content was significantly different between cadaver treatments and controls ($p<0.001$, ANOVA, TukeyHSD) but not between hanging and ground cadavers or between fake pigs and control ($p>0.9$, ANOVA, TukeyHSD).

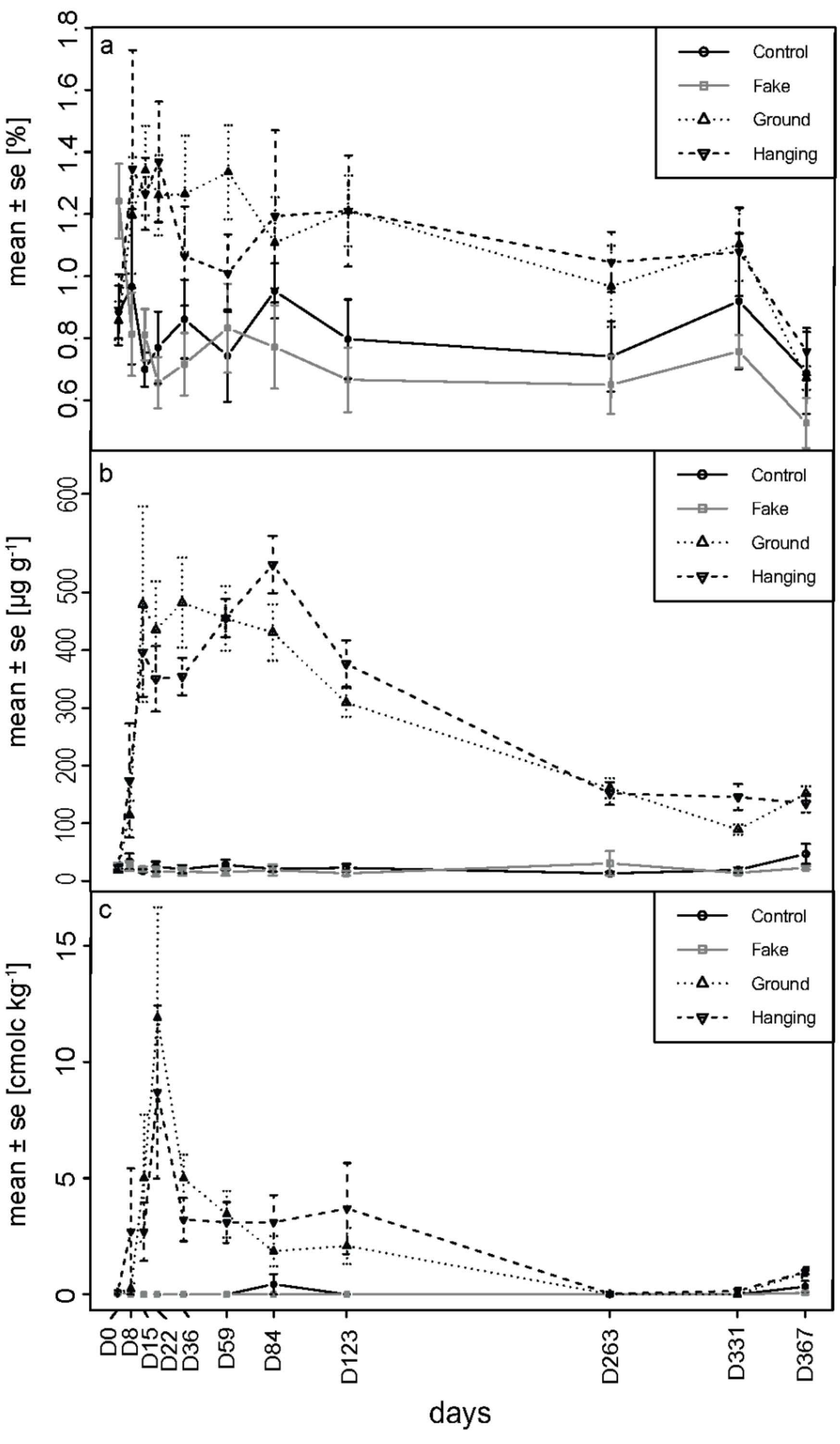

Figure 4. Average ± SE for total Nitrogen (N) concentration [%] (a), bioavailable Phosphorous (P_{bio}) content [µg g^{-1}] (b) and Potassium (K^+) content [$cmol_c$ kg^{-1}] (c) in the

control, fake pig, ground pig and hanging pig treatments over time at the Bois-du-Clos spruce forest experimental site (Neuchâtel, Switzerland).

3.8. Carbon (C)

Soil carbon content ranged from 8.51 to 36.54 % in the control, 5.8 to 35.31 % in the fake, 9.01 to 31.97 % in the ground and 8.78 to 36.68 % in the hanging pig cadavers treatment (Table 2). No significant differences between the four sets of samples were observed on any of the sampling days ($p > 0.05$, ANOVA, TukeyHSD; Fig. S2).

3.9. Redundancy analysis (RDA)

The RDA of the chemical variables (response variables) in function of time (sampling days) and treatments (explanatory variables) showed a clear difference between the cadaver treatments and the controls (axis 1) as well as temporal changes (axes 1 and 2) (Fig. 5). The ground and hanging cadavers' samples diverged from the control samples from T1 onwards (Fig. 5). Variables most strongly correlated with axis 1 and thus best explaining the difference between cadaver and control samples were P, NH_4^+, total N and K^+. Starting from D263-D367, cadaver-impacted samples started to converge back towards the control and fake pig samples. However, by T10 they clearly remained different from the control and fake pigs, owing mainly to higher nitrate concentrations.

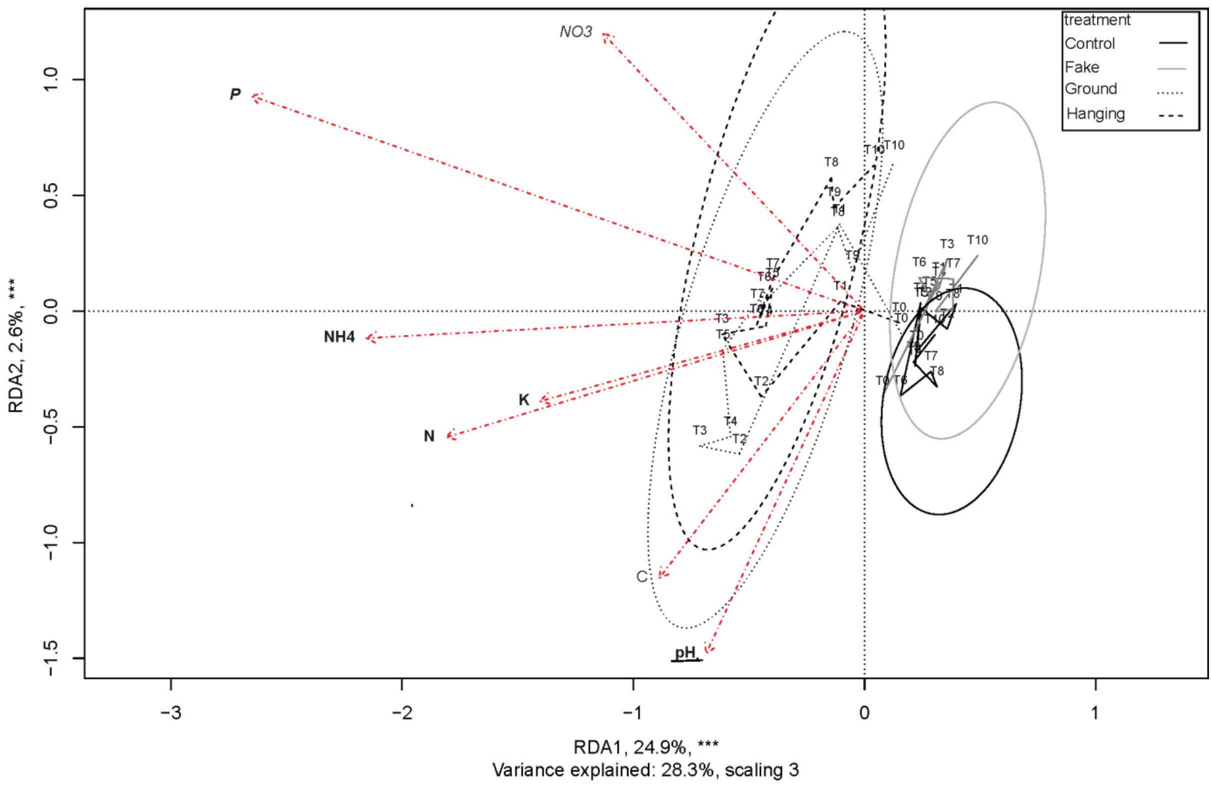

Figure 5. Redundancy analysis (RDA) ordination diagram showing the response of soil chemistry according to treatment (control, fake pig, ground pig and hanging pig treatments) and time in a spruce forest at the Bois-du-Clos experimental site (Neuchâtel, Switzerland). The lines (solid black: control; solid grey: fake; dotted: ground pig; dashed: hanging pig) join the centroids of the five replicates from each sampling day. T0 to T10 represent the mean coordinates of the 5 replicates per treatment (numbers indicating the time since death in days). For better readability D0 was represented by T0, D8 by T1, D15 by T2 and so forth until D367 by T10. Arrows represent the chemical i.e. explanatory variables i.e. NO_3, P, NH_4, K, N, C, and pH. Ellipses show the SD of the mean position of every treatment (solid black: control; solid grey: fake; dotted: ground pig; dashed: hanging pig). The grouping of the chemical markers is indicated by different font styles: EPM (bold), LPM (italic), EPM+ LDM (bold/underlined), and EPM+LPM (bold/italic).

3.10. Grouping according to EPM, LPM and LDM

Seven chemical soil markers (pH, NH_4^+, NO_3^-, N, C, P, K^+) were investigated in all treatments and at all time points. The turning point from early (</= D59) to late markers (> D59 - </= D367) in our study is two months after the cadavers were placed, which is after the greatest mass loss (Fig. 2) and the end of the main pulse of cadaveric fluids into the soil (after advanced decay) (Fig. 1). Based on significant differences between controls and cadaver treatments, chemical markers were grouped into three categories: early peak markers (EPM), late peak markers (LPM) and late decrease markers (LDM) (Table 3, Figure 5). As some chemical markers could be attributed to more than one category, in this analysis five groups of markers could be identified:

EPM: Nitrogen and potassium

LPM: Nitrate

EPM and LDM: pH

EPM with continuing elevated levels (ELs): Ammonium

EPM and LPM followed by EL: Phosphorous

No category: Carbon

No (+) RL (reduced levels) could be assigned.

4. Discussion

In both cadaver treatments mass loss followed a sigmoidal pattern in line with the classical pattern of breakdown of cadaver tissue and release of fluids taking place at the beginning of the decomposition process (Carter et al., 2007; Spicka et al., 2011). The longer active decay stage in the hanging cadavers was due to a lower insect activity (especially beetles) on the hanging cadavers (unpublished data) and the continuous dripping and loss of maggot masses from the hanging cadavers. However, overall in this study soil chemistry between ground and hanging cadavers did not reveal significant differences.

At the beginning of the experiment (>D15) soil pH, NH_4^+, N, P and K^+ (EPMs) increased in at least one of the two cadaver treatments. On D15 all cadavers were in the active decay stage, skin was ruptured and cadaveric fluids were released into the soil. The observed pattern is in line with the documented release of C-, N- and P-based products into the soil due to proteins, lipids and carbohydrates degradation from vertebrate cadavers (Stokes et al., 2009b).

During these processes an increase of soil pH in our study was observed beneath the ground cadavers as compared to the controls. In previous studies, soil pH has been shown to either decrease and increase beneath human and other mammal remains (Aitkenhead-Peterson et al., 2012; Benninger et al., 2008). In our study the increase of pH is probably due to an accumulation of ammonium- ions that follow the same pattern as shown by Benninger et al. (2008). Therefore, pH and NH_4^+ can be regarded as EPMs. It is suggested that during and after the release of cadaveric fluids the soil beneath cadavers becomes more and more anoxic for a while, which would explain why NH_4^+ ions were not further nitrified (Aitkenhead-Peterson et al., 2015).

Although pH beneath the hanging cadavers was also elevated at the beginning, it did not reach the significant values from the ground pig treatment. The dripping of the fluids and maggot masses probably did not cause a complete temporary shift to anoxia and did not cover the area beneath the cadaver completely. This would have allowed some nitrification to take place. The significant decrease of pH towards the end of the experiment in both cadaver treatments is line with the decline of NH_4^+ after >2 months and an increase of NO_3^-. This groups pH additionally into LDMs and NO_3^- into LPMs. It suggests a return of aerobic conditions allowing aerobic nitrification after an initial lag phase (Aitkenhead-Peterson et al., 2015; Stokes et al., 2013). This follows a pattern shown by Meyer at al. (2013) for $NH_4^+$and NO^{3-}, who suggested that ammonification is the dominant process up to advanced decay and nitrification after advanced decay. Significantly elevated NO_3^- was described after one and three years beneath decomposing pig cadavers (Anderson et al., 2013).

In our study, total N (EPM) increased two and three weeks after the beginning of the experiment in the cadaver treatments. Similar findings were observed by Benninger et al. (2008) showing an increase of total N in the first 14 days of the decomposition trial and smaller peaks between days 21 and 42, and could be either the influx of organic or inorganic nitrogen forms. This is not surprising as a cadaver is a rich source for N for instance 26g kg^{-1} N concentration is reported for pigs (Benninger et al., 2008). The main N from cadavers derives from the breakdown of proteins, this process does not occur at a uniform rate and the degradation products can be released over a longer time- span including more decomposition stages (Macdonald et al., 2014). It might not be straightforward to group N into EPMs alone because other studies have shown that total N was significantly higher after one year beneath decomposing pigs (Anderson et al., 2013; Parmenter and MacMahon, 2009). Here more data will be necessary.

Although carbon accounts for 20% of the mass of cadavers (Carter et al., 2007) no significant changes were observed in the soil beneath the cadavers, which is in line with other studies (Anderson et al., 2013; Benninger et al., 2008; Meyer et al., 2013). One reason for this might be that the intense pulse of C input caused an increase in micro-organisms that utilize carbon and then release CO_2 into the atmosphere via respiration. Nevertheless, results are conflicting and some studies describe significant increases in total carbon beneath decomposing cadavers (Macdonald et al., 2014).

The input of P from cadavers, where P is stored in proteins, coenzymes, sugar phosphates and phospholipids (Dent et al., 2004), may translate into a large increase in soil as available P (Perrault and Forbes, 2016). In our study, bioavailable P peaked at the beginning of the experiment (EPM) but also on day 84 (LPM) and showed significantly elevated levels until the end of the experiment ((+) EL in the cadaver treatments when compared to the controls). Therefore, it cannot be assigned to just one category. Our results are in line with previous studies: The presence of a double peak was also noted by Benninger et al. (2008) and Perrault and Forbes (2016). Additionally, MacDonald et al. (2014) described a significant and lasting increase in plant available P relative to the control 12 and 24 weeks after carcass addition and extractable P concentrations were described to be higher at carcass-impacted sites than in the surrounding soil one and three years postmortem (Towne, 2000). Phosphorous concentration seems to be a good indicator for locating the decomposition of remains (Perrault and Forbes, 2016).

Potassium was also grouped into the EPMs. Assuming that 100 g of pig body tissue contain approximately 280 mg K (Spray and Widdowson, 1950) being released into the soil relatively early in the decomposition process when tissues are broken down. Elevated K levels were also reported by Aitkenhead-Peterson et al. (2012) and Stokes et al. (2009a; 2013) beneath decomposing cadavers and buried skeletal muscle tissues respectively.

5. Conclusion

The results from this and other studies indicate that it might be possible to categorize soil chemical markers according to their response pattern to decomposition products over time. As this is the first attempt to group cadaver-impacted soil chemical markers, we correlated the changes to decomposition stages and weight loss of the cadavers. A grouping into defined markers can be highly useful when the changes in soil chemistry are related to changes in the composition of soil organism communities. When applied in a forensic context a marker that shows clear and high peaks and/or decreases for a short period of time might be more useful than a marker that has elevated levels over a longer time-span to estimate the PMI. Chemical markers may thus be a useful addition to the forensic research toolkit when investigating homicides or other unclear death cases.

Conflict of interest

No conflict of interest declared.

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Table 1.

reference	cadavers	time span/year	sampling days	country	pH	Ammonium	Nitrate	Nitrogen	Phosphorous	Potassium	Carbon
Aitkenhead-Peterson et al., 2012	2 human bodies	2009-2010	288 (corpse 1) and 248 (corpse 2) days after	Texas, USA	lower (p<0.001)	–	–	higher (p<0.001)	higher (p<0.001)	higher (p<0.001)	higher (p<0.001)
Anderson et al., 2013	3 (2005) + 3 pigs (2007)	2005-2010	1 and 3 years after	Nebraska, USA	lower (1 year; p<0.05)	–	higher (1 + 3 years, p<0.05)	higher (p<0.05) after 1 year	–	–	–
Barton et al., 2016	12 kangaroos	2010-2015	5 years after	Canberra, Australia	–	–	–	–	higher (p<0.015)	–	–
Benninger et al., 2008	5 pigs	100 days (2006)	weekly (first 6 weeks), monthly after	Ontario, Canada	higher (D14, D23,D43; p<0.05)	–	–	–	–	–	–
					lower (D30, D72, D100; p<0.05)						
Carter et al. , 2008	juvenile rats	28 days	7,14,21,28 days after	Queensland, Australia	higher (D7- D28; p<0.001)	–	–	–	–	–	–
Cobaugh et al., 2015	4 human bodies	summer, autumn, 2012	up to 198 days after	Tennessee, USA	–	–	–	higher (p<0.05)	–	–	higher (p<0.05)
Hopkins et al., 2000	3 pigs	1996-1998	430 days after	England	elevated levels†	elevated levels	–	elevated levels	–	–	elevated levels
Macdonald et al., 2014	18 kangaroos	2010	0, 12, 24 weeks after	Canberra, Australia	higher (week 12, 24; p<0.001)	higher (week 12, 24; p<0.001)	–	higher (week 12, 24; p<0.001)	higher (week 12, 24; p<0.001)	–	higher (week 12; p<0.001)
Melis et al., 2007	6 bisons	1997-2004	summer 2004	Poland	higher (1 to 6 years; p<0.0001)	–	higher (1 year, p<0.001)	–	–	–	–
Metcalf et al., 2016	120 mice‡	71 days	0,3,6,9,14,29,44,70 days after	Colorado, USA	higher (p<0.05)‡	higher (p<0.05)‡	higher (p<0.05)‡	higher (p<0.05)‡	–	–	–
Meyer et al., 2013	6 pigs	winter, 2008-2010	0,15,30,60 days after	Nebraska, USA	higher (D60; p<0.001)	higher (D60; p<0.05)	higher (D60; p<0.05)	higher (D60; p<0.05)	–	–	–
		summer, 2008-2010			higher (D15; p<0.05)	higher (D15-D60, p<0.001)	higher (D15 (p<0.05)- D60(p<0.001))	higher (D30 (p<0.05), D60 (p<0.001))			
					lower (D60;p<0.001)						
Parmenter&MacMahon, 2009	various vertebrates‡	all seasons, 3 years	15, 27, 39 months	Wyoming, USA	–	–	–	higher (first and second year)†/‡	–	higher†/‡	–
Stokes et al., 2009a	skeletal muscle tissue (pork)	37 days	2,4,6,8,12,16,23,30,37 days after	WA, Australia	higher (from D2; p<0.001)	higher (from D2; p<0.001)‡	higher (from D16; p<=.001)‡	–	–	higher (from D2; p<0.001)	–
Stokes et al., 2013	skeletal muscle tissue	37 days	2,4,6,8,12,16,23,30,37 days after	WA, Australia	higher (from D2)‡	higher (from D2-D16/23)‡	higher (from D8/D12)‡	–	–	higher (from D2)	–
	(human, pork, beef, lamb)				lower (from D23)‡						
Towne, 2000	bison, cattle, deer	5 years	yearly	Kansas, USA	lower (p<0.01)‡	–	–	higher (1, 2 years after; p<0.05)	higher (1-3 years after; p<0.05)	–	–

† no significance given

‡ see reference for details

Table 1. Overview of selected studies on vertebrate cadaver decomposition and its effects on defined chemical markers in soil. Unless indicated, only significant differences are shown for the cadaver impacted soils in comparison to controls ("days, weeks, months, years after" refers to time elapsed since the beginning of the experiment i.e. the placing of the cadavers).

Table 2.					
		control	fake pig	ground pig	hanging pig
pH	mean ± [SE]	6.1 ± [0.08]	5.58 ± [0.05]	6.5 ± [0.18]	5.95 ± [0.16]
	min	5.05	4.71	4.63	4.68
	max	7.02	6.5	8.76	8.7
NH4+ [µg g-1]	mean ± [SE]	12.57 ± [1.4]	16.04 ± [2.03]	391.88 ± [54.84]	316.7 ± [45.88]
	min	0.92	1	1.98	0.64
	max	50.57	62.51	1561.78	1124.71
NO3- [µg g-1]	mean ± [SE]	14.82 ± [1.63]	24.52 ± [5.07]	41.42 ± [6.8]	39.87 ± [4.85]
	min	3.12	3.36	3.7	3.67
	max	57.26	235.89	321.97	164.35
N [%]	mean ± [SE]	0.82 ± [0.04]	0.77 ± [0.04]	1.12 ± [0.05]	1.11 ± [0.06]
	min	0.45	0.31	0.58	0.57
	max	1.95	1.55	1.81	2.78
C [%]	mean ± [SE]	16.51 ± [0.85]	15.53 ± [0.87]	17.95 ± [0.71]	17.62 ± [0.78]
	min	8.51	5.8	9.01	8.78
	max	36.54	35.31	31.97	36.68
P [µg g-1]	mean ± [SE]	24.39 ± [2.64]	19.89 ± [2.5]	284.29 ± [29.58]	283.03 ± [25.11]
	min	4.64	0.56	10.96	13.77
	max	110.86	114.41	1105.3	724.42
K [cmolc kg-1]	mean ± [SE]	0.08 ± [0.05]	0.01 ± [0.01]	2.78 ± [0.66]	2.59 ± [0.55]
	min	0	0	0	0
	max	2.2	0.34	30.76	22.93

Table 2. Chemical components in the control, fake pig, ground pig and hanging pig treatments over the course of the experiment at the Bois-du-Clos spruce forest experimental site (Neuchâtel, Switzerland) showing mean and standard error (SE), minimum (min) and maximum value (max).

Table 3.												
	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	
days	0	8	15	22	36	59	84	123	263	331	367	Figures
pH	–	–	EPM	EPM	EPM	–	–	–	LDM	LDM	LDM	Fig. 3a
NH4+	–	–	EPM	EPM	EPM	EPM	(+) EL	(+) EL	–	–	–	Fig. 3b
NO3-	–	–	–	–	–	–	–	–	LPM	LPM	LPM	Fig. 3c
N	–	–	EPM	EPM	–	–	–	–	–	–	–	Fig. 4a
P	–	–	EPM	EPM	EPM	EPM	LPM	(+) EL	(+) EL	(+) EL	(+) EL	Fig. 4b
K	–	–	–	–	EPM	EPM	–	–	–	–	–	Fig. 4c
C	–	–	–	–	–	–	–	–	–	–	–	Fig. S2

Table 3. Grouping of chemical components into EPM (early peak marker), LPM (late peak marker), LDM (late decrease marker), +EL (+ elevated levels). The grouping of the chemical markers is indicated by different font styles: EPM (bold), LPM (italic), EPM+ LDM (bold/underlined), and EPM+LPM (bold/italic).