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

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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 postmortem interval. However, cadaver impacts on soils are rarely studied, making it difficult to identify common patterns.

We investigated the effects of decomposing pig cadavers (*Sus scrofa domesticus*) on soil chemistry (pH, ammonium, nitrate, nitrogen, phosphorous, potassium and carbon) over a one-year period in a spruce-dominant 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. For actual casework further studies and more data are necessary to refine the marker categories along a more precise timeline and to develop a method that can be used in court.

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%) [1]. 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 [2]. Cadavers are nutrient-rich [3] and during decomposition, they release large amounts of water and breakdown products including proteins, fats and carbohydrates, which enter the underlying soil [4] and have a major impact on soil organisms [1,5,6]. Understanding these effects is relevant for both soil ecology and forensic taphonomy and may help us develop new tools for the estimation of a postmortem interval (PMI) i.e. the time elapsed since death [7,8].

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

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remains [9]. Nevertheless, decomposition is a time-continuous process with overlapping and not clear-cut stages [10]. Various abiotic and biotic factors can influence decomposition and accordingly its impact on soils. These factors include or may include temperature [11,12], moisture [7], pH [13], soil type [14], season [15], access by insects [16], vertebrate scavenging [17], associated material e.g. clothing [18], burial [19], trauma (open wounds) [20], size, age and type of carcass [21–23].

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 [24,25], the concentration of ammonium [15,26], nitrates [15,27], total nitrogen [2,27], total carbon [28,29], phosphorous [23,29], potassium [22,24], magnesium [24] and calcium [24,30] (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 [31].

The impact of pig cadavers on selected soil chemical markers was therefore investigated over a one-year period to include seasonal variation and to monitor the changes in soil chemistry beyond the peak decay stages. The effects of pig cadavers that were placed directly on the ground and pig cadavers that were hung one metre aboveground on soil chemistry were compared and contrasted with two controls (bare soil and bags filled with soil). The 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, 775 m a.s.l.). The study site was 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 Ref. [5] (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). The so-called fake pig treatment was chosen to discriminate the effects of cadaveric fluids from the effects of a changed microclimate e.g. reduced evaporation, no direct sunlight, higher moisture content caused by the soil filled bag that was placed on the surface.

Ten domestic pigs (*Sus scrofa domesticus*), 8 females and 2 males, 10 weeks old, were bought from a local farm. In a variety of studies domestic pigs were used as surrogate for humans and considered to be excellent models [37]. The sampling set-up using pig cadavers of more or less the same weight and age allowed us to compare repetitive sampling of the experimental units. The domestic pigs 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. (Note: To our knowledge, effects of the above-mentioned substances on the rate of decomposition have not yet been studied.) The pigs showed no visible wounds or injuries. The average cadaver weight was 27.8 kg ± 0.8 kg (SE). All cadavers were placed in cages (140 cm × 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 [9] 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 (more than 9 days).

On 11 sampling days from June 2013 until July 2014, a total of 220 soil samples (11 days × 4 treatments × 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 × 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 [38]. Ammonium (NH_4^+) and nitrate (NO_3^-) analyses were performed directly after sampling using colorimetric determination (Biochrom Libra S11 Spectrophotometer) [39]. 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 [40]. 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). Potassium was selected from the elemental analysis technique (that was used to quantify K^+, Mg^{2+}, Ca^{2+}, Na^{2+} and Al^{3+}) as it was most suitable for our marker system (see Section 2.4) for the time span of one year. 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) showed significantly higher concentrations in the soil beneath cadavers when compared to the

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).

Ref.	Cadavers	Time span/ year	Sampling days	Country	pH	Ammonium	Nitrate	Nitrogen	Phosphorous	Potassium	Carbon
[24]	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 ($p < 0.001$)
[27]	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	–	–	–
[31]	12 kangaroos	2010–2015	5 years after	Canberra, Australia	–	–	–	–	Higher ($p < 0.015$)	–	–
[25]	5 pigs	100 days (2006)	Weekly (first 6 weeks), monthly after	Ontario, Canada	Higher (D14, D23, D43; $p < 0.05$) Lower (D30, D72, D100; $p < 0.05$)	–	–	–	–	–	–
[12]	Juvenile rats	28 days	7, 14, 21, 28 days after	Queensland, Australia	Higher (D7-D28; $p < 0.001$)	–	–	–	–	–	–
[32]	4 human bodies	Summer, autumn, 2012	Up to 198 days after	Tennessee, USA	–	–	–	Higher ($p < 0.05$)	–	–	Higher ($p < 0.05$)
[28]	3 pigs	1996–1998	430 days after	England	Elevated levels[a]	elevated levels	–	Elevated levels	–	–	Elevated levels
[29]	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; $p < 0.001$)
[30]	6 bisons	1997–2004	Summer 2004	Poland	Higher (1–6 years; $p < 0.0001$)	–	Higher (1 year, $p < 0.001$)	–	–	–	–
[33]	120 mice[b]	71 days	0, 3, 6, 9, 14, 29, 44, 70 days after	Colorado, USA	Higher ($p < 0.05$)[b]	higher ($p < 0.05$)[b]	Higher ($p < 0.05$)[b]	Higher ($p < 0.05$)[b]	–	–	–
[15]	6 pigs	Winter, 2008–2010 Summer, 2008–2010	0, 15, 30, 60 days after	Nebraska, USA	Higher (D60; $p < 0.001$) Higher (D15; $p < 0.05$) Lower (D60; $p < 0.001$)	higher (D60; $p < 0.05$) higher (D15-D60, $p < 0.001$)	Higher (D60; $p<0.05$) Higher (D15 ($p < 0.05$)-D60 ($p < 0.001$))	Higher (D60; $p < 0.05$) Higher (D30 ($p < 0.05$), D60 ($p < 0.001$))	–	–	–
[2]	Various vertebrates[b]	All seasons, 3 years	15, 27, 39 months	Wyoming, USA	–	–	–	Higher (first and second year)[a/b]	–	Higher[a/b]	–
[34]	7 pigs	3 months (2 trials), 2011	In decreasing intervals[b]	Ontario, Canada	Lower ($p < 0.05$)[b]	–	–	–	Higher ($p < 0.05$)[b]	Not significant	–
[26]	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; $p < 0.001$)[b]	Higher (from D16; $p <= 0.001$)[b]	–	–	Higher (from D2; $p < 0.001$)	–
[22]	Skeletal muscle tissue (Human, pork, beef, lamb)	37 days	2, 4, 6, 8, 12, 16, 23, 30, 37 days after	WA, Australia	Higher (from D2)[b] Lower (from D23)[b]	higher (from D2-D16/23)[b]	Higher (from D8/D12)[b]	–	–	Higher (from D2)	–
[23]	Bison, cattle, deer	5 years	Yearly	Kansas, USA	Lower ($p < 0.01$)[b]	–	–	Higher (1, 2 years after; $p < 0.05$)	Higher (1–3 years after; $p < 0.05$)	–	–
[35]	5 pigs (surface trial)	97 days	Daily (until day 10), every two days (day 11–16) Then weekly until day 97	Ontario, Canada	–			Ninhydrin reactive nitrogen (NRN) Higher (D3-D97; $p < 0.05$)			
[36]	7 human bodies	1988–1989	Every 3 days (spring & summer) Weekly (autumn & winter)	Tennessee, USA	Elevated levels[a]	Elevated levels[a]	–	–	–	Elevated levels[a]	–

[a] No significance given.
[b] See reference for details.

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) showed 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) showed 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 were followed by a relatively fast decrease/increase and levels discontinued being significantly higher or lower than the controls, markers were named EPM, LPM, LDM without any addition. In the case where peaks or decreases continued to be significantly higher/lower than the controls over a certain period of time either (+) EL (elevated levels) or (−) RL (reduced levels) were 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 follow the parametric assumptions of a normal distribution, variables were transformed (log 10, decostand) before the analyses. Normality was checked graphically following Gravetter and Wallnau [41], before and after transformation.

To test the significance of differences between treatments at each sampling day we first performed an analysis of variance (ANOVA) followed by a Tukey's post hoc analysis (TukeyHSD) when necessary, considering that each treatment was independent from the others (see Supplementary Table S1). Secondly, we performed a linear mixed-effects model (lme) which includes a nested random effect taking the repeated measures over time into account, to specifically test the difference for each treatment between sampling dates. We, then, assessed the significance of the difference over time by using one-way ANOVA with repeated measure and post hoc multiple comparison of means (Tukey contrasts, when necessary) with Bonferroni adjusted p-value (see Supplementary Table S2).

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) [42], and packages vegan, version 2.4.1 [43], nlme, version 3.1-128 [44], multcomp, version 1.4-6 [45] and lme4, version 1.1-12 [46].

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

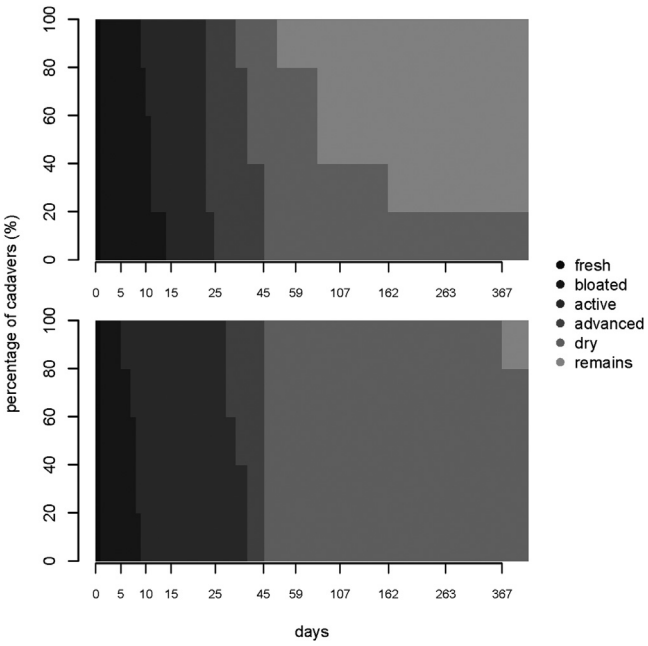

Fig. 1. Duration of decomposition stages, and percentage of cadavers representing a given decomposition stage in the ground (top) and hanging pig (bottom) cadaver treatments over time at the Bois-du-Clos spruce forest experimental site (Neuchâtel, Switzerland). Decomposition stages are shown in different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

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).

3.2. Soil pH

Soil pH beneath the control and fake pigs fluctuated in a range of 2 units over the one-year period (Table 2,Fig. 3a). In contrast, pH beneath the ground cadavers increased by 4 units (Table 2, Fig. 3a) and was significantly different in comparison to the control and fake pig samples from days 15 to 36 (for detailed p-values see Supplementary material Table S1). Additionally, it was significantly higher to the hanging cadavers samples on D22 (adjusted p-value: 0.004) (Table S1). This increase was followed by a decrease reaching significantly lower pH values as compared to the control from D263 to D367 (adjusted p-values: 0.022, 0.019, 0.003 respectively) (Table S1).

In comparison, the increase in pH beneath the hanging cadavers at the beginning of the experiment was weaker (Fig. 3a), but the decrease towards the end of the experiment (D263-367) was also significant when compared to the control (adjusted p-value: <0.001 for all time points) and the fake pig treatment (adjusted p-value: 0.01, 0.01, 0.03 respectively) (Table S1).

3.3. Ammonium (NH_4^+)

Overall ammonium content differed significantly between cadaver treatments and controls (adjusted p-value: <0.001) but not between hanging and ground cadavers (adjusted p-value: 0.97) or between fake pigs and control (adjusted p-value: 0.89).

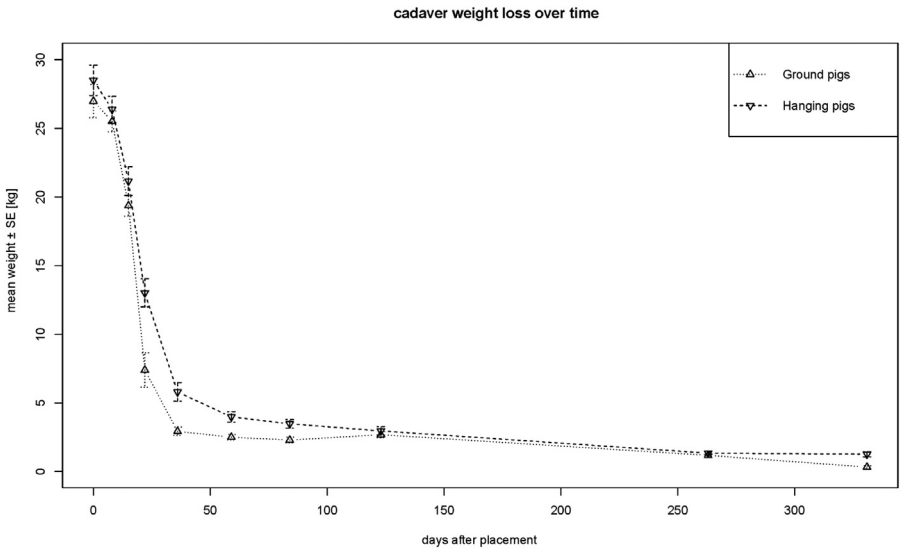

Fig. 2. Average cadaver weight loss ± SE [kg] in the ground and hanging pig cadaver treatments over time at the Bois-du-Clos spruce forest experimental site (Neuchâtel, Switzerland).

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).

		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
NH_4^+ [$\mu g\,g^{-1}$]	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
NO_3^- [$\mu g\,g^{-1}$]	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 [$\mu g\,g^{-1}$]	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

Ammonium content in the soil of the control and fake pig samples varied slightly within almost the same range (Table 2), but there was a huge and significant increase in ammonium content in the ground and hanging pig samples from D15 to D123 with a peak on D59 in contrast to both controls (adjusted p-values: always <0.001) (Table S1, 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 (adjusted p-values: >0.05) (Table S1, Fig. 3b).

3.4. Nitrate (NO_3^-)

Overall nitrate content differed significantly between cadaver treatments and controls (adjusted p-values: ground — control <0.001, ground — fake: 0.003, hanging — control: <0.001, hanging — fake: 0.004) but not between hanging and ground cadavers (adjusted p-value: 1) or between fake pigs and control (adjusted p-value: 0.4) (Table S1). Although fluctuations of the soil nitrate content in the control, the fake pig, the ground and the hanging pig samples were observed (Table 2, Fig. 3c), no significant differences were recorded between the treatments until D263, except on D8 between the ground and fake pig treatment (adjusted p-value: 0.05, Table S1). On D263 and on D367 ground cadaver samples were significantly different from both controls (adjusted p-values control: <0.001, 0.003 and fake: 0.002, 0.007 respectively) and hanging cadavers samples accordingly on D263, D331 and D367 (adjusted p-values control: <0.001, 0.03, <0.001 and fake: <0.001, 0.03, <0.001 respectively) (Table S1).

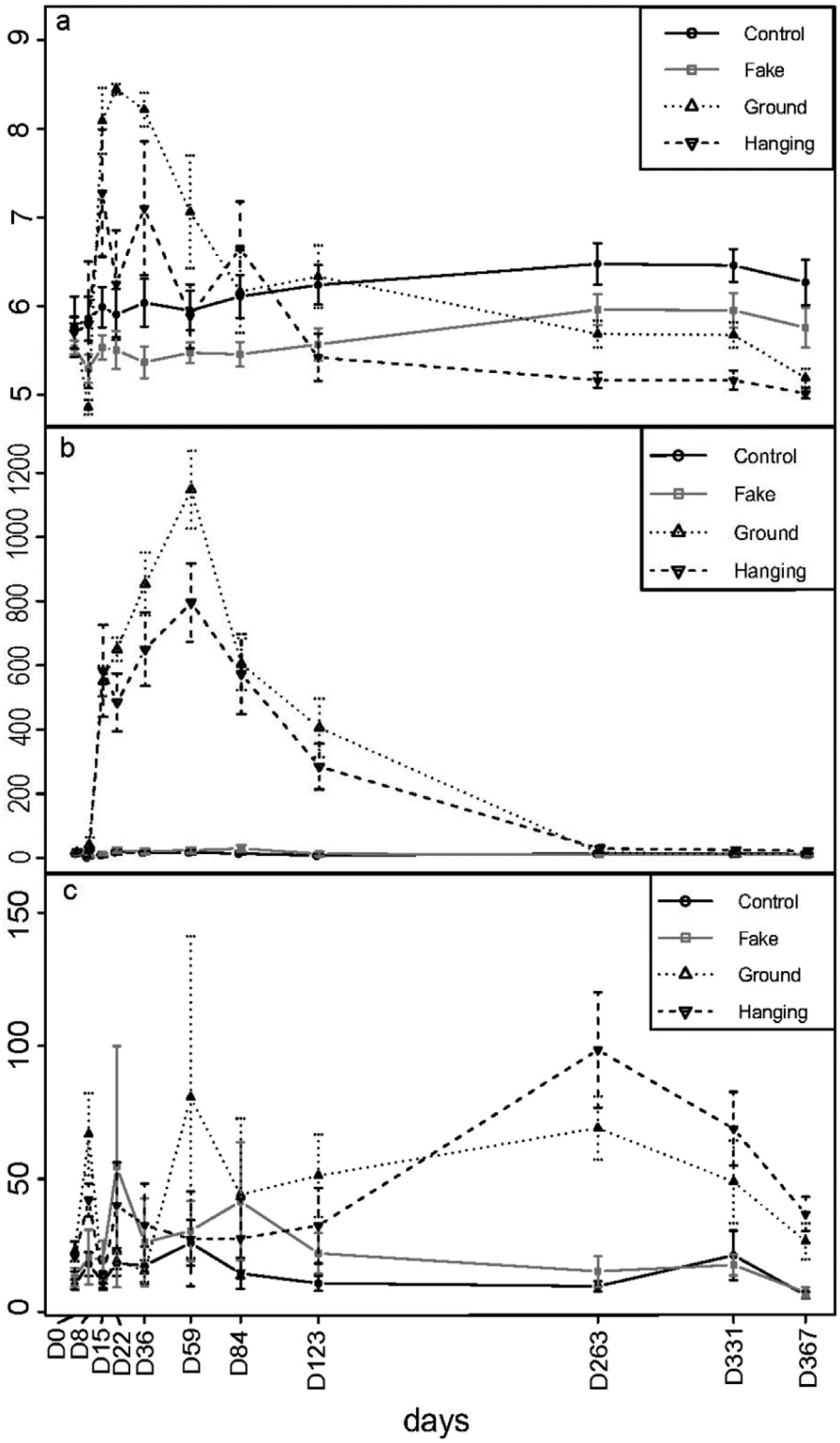

Fig. 3. Average ± SE for pH (a), Ammonium (NH_4^+) content [$\mu g\, g^{-1}$] (b) and Nitrate (NO_3^-) [$\mu g\, g^{-1}$] (c) in the control, fake pig, ground pig and hanging pig treatments over time at the Bois-du-Clos spruce forest experimental site (Neuchâtel, Switzerland).

3.5. Nitrogen (N)

Overall nitrate content differed significantly between cadaver treatments and controls (adjusted p-values: <0.001, for all comparisons) but not between hanging and ground cadavers (adjusted p-value: 0.99) or between fake pigs and control (adjusted p-value: 0.68). In the soil samples from beneath the ground and hanging cadavers nitrogen content increased at the beginning of the experiment (Table 2, Fig. 4a) and was significantly higher as compared to both controls on D15 and D22 (adjusted p-values ground — control: 0.001 and 0.05, ground — fake: 0.01 and 0.009, hanging — control: 0.003 and 0.02, hanging — fake: 0.02 and 0.004 respectively) (Table S1). Nitrogen

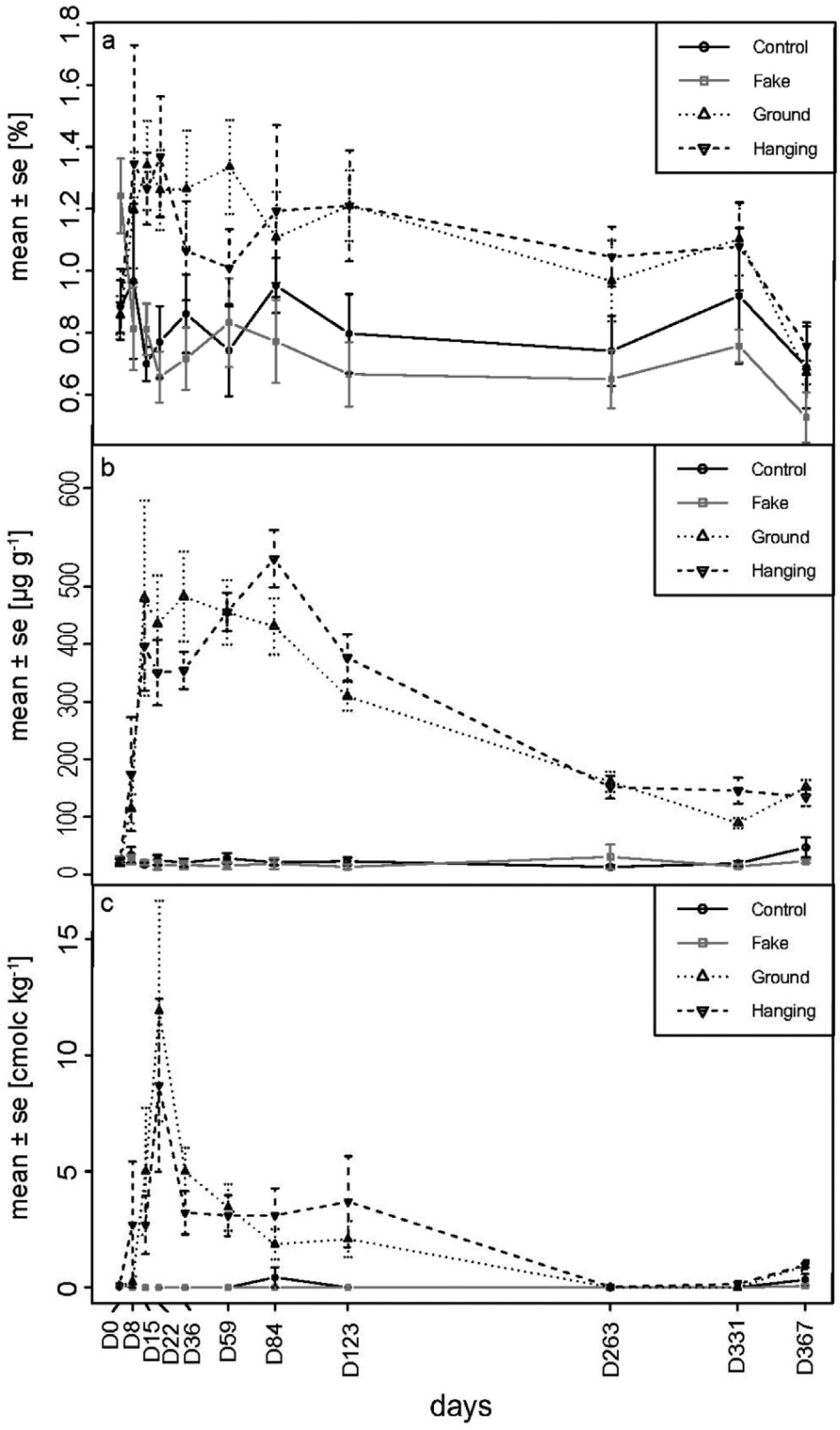

Fig. 4. Average ± SE for total Nitrogen (N) concentration [%] (a), bioavailable Phosphorous (P_{bio}) content [$\mu g\, g^{-1}$] (b) and Potassium (K^+) content [$cmol_c\, kg^{-1}$] (c) in the control, fake pig, ground pig and hanging pig treatments over time at the Bois-du-Clos spruce forest experimental site (Neuchâtel, Switzerland).

content in the cadaver samples stayed above the controls until D331, not significantly and without any clear pattern (Fig. 4a, Table S1).

3.6. Bioavailable phosphorous (P_{bio})

Overall bioavailable phosphorous content differed significantly between cadaver treatments and controls (adjusted p-values: <0.0001 for all comparisons) but not between hanging and ground pigs or between fake pigs and control (adjusted p-values: 1 and 0.36 respectively). Phosphorous content in soil varied slightly in the control and in the fake pig samples over the course of the experiment (Table 2; Fig. 4b). Phosphorous content started to increase in the early phase of decomposition and on D8 both cadaver treatments were significantly different from the control (adjusted p-values: 0.03 for

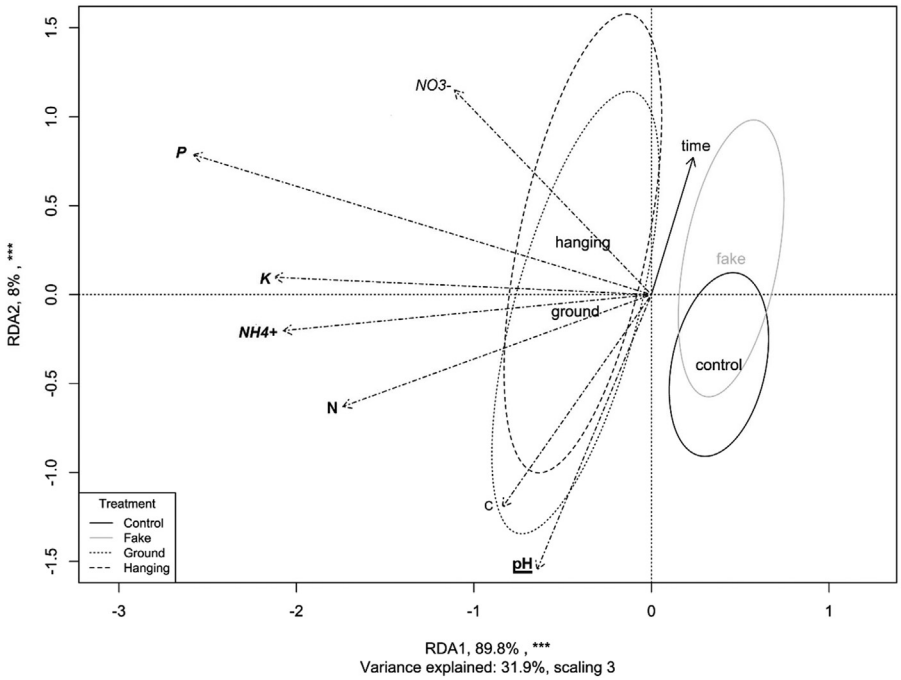

Fig. 5. Redundancy analysis (RDA) ordination diagram showing the response of soil chemistry according to treatment (control, fake pig, ground pig and hanging pig) and time in a spruce forest at the Bois-du-Clos experimental site (Neuchâtel, Switzerland). Dashed arrows represent the explanatory variables i.e. chemical variables NO_3^-, P, K, NH_4^+, N, C, and pH. The plain arrow and the ellipses (treatments) represent the projection of the selected constrained parameters. Ellipses represent the standard deviation from the mean position of every treatment (solid black: control; solid grey: fake; dotted: ground pig; dashed: hanging pig). The main grouping of the chemical markers is indicated by different font styles: EPM (bold), LPM (italic), EPM+ LDM (bold/underlined), and EPM + LPM (bold/italic).

both). Additionally on D8 the ground pig treatment was different from the fake pig (adjusted p-values: 0.03) (Table S1, Fig. 4b), whereas the difference between hanging and fake pig treatment was not significant. This was followed by a huge 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) (adjusted p-values: <0.001 for all) (Table S1; Fig. 4b). Although phosphorous decreased again after the second peaks, the content stayed significantly higher until the end of the experiment (D367) (adjusted p-values: <0.001 for all) (Table S1, Fig. 4b).

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

Overall potassium content was significantly different between cadaver treatments and controls (adjusted p-values: <0.001 for all comparisons) but not between hanging and ground pigs (adjusted p-value: 1) or between fake pigs and control (adjusted p-value: 0.94). Potassium content in the control and fake pig samples did not change over the course of the experiment (Table 2, 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 D15 until D123 and on D367 (adjusted p-values: range from <0.001 to 0.05 for all permutations, for details see Table S1).

3.8. Carbon (C)

The range of carbon content was more or less the same for all four treatments (Table 2). No significant differences between the four sets of samples were observed on any of the sampling days (Table S1, Fig. S2).

3.9. Redundancy analysis (RDA)

The redundancy analysis (RDA, Fig. 5) allowed us to project the chemical variables in a space defined by the treatments (as factors) and the elapsed time. The selected explanatory variables explained 31.9% of the RDA. Axis 1 was correlated with the treatments (control and fake vs. ground and hanging pigs) and explained 89.8% of the variance. Axis 2 represented the elapsed time of the overall experiment and explained 8% of the variance. Both axes were significant (p-value <0.001). The RDA showed a clear difference between the two cadaver treatments and the controls (axis 1) as well as temporal changes (axis 2). 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^+. C, pH, and, to a lesser extent, NO^3 were correlated with the elapsed time over the course of the experiment.

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 was two months after the cadavers were placed, which was 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, Fig. 5). As some chemical markers could be attributed to more than one category, in this analysis five groups could be identified:

1. EPM followed by EL: Nitrogen
2. LPM: Nitrate
3. EPM and LDM: pH
4. EPM and LPM: Ammonium, phosphorous, potassium
5. No category: Carbon

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

Table 3
Grouping of chemical components into EPM (early peak marker), LPM (late peak marker), LDM (late decrease marker). The grouping of the chemical markers is indicated by different shades of grey: EPM (light grey), LPM (dark grey), LDM (grey).

	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
NH_4^+			EPM	EPM	EPM	EPM	LPM	LPM				Fig. 3b
NO_3^-									LPM	LPM	LPM	Fig. 3c
N			EPM	EPM								Fig. 4a
P		EPM	EPM	EPM	EPM	EPM	LPM	LPM	LPM	LPM	LPM	Fig. 4b
K^+			EPM	EPM	EPM	EPM	LPM	LPM			LPM	Fig. 4c
C												Fig. S2

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 [1,21]. 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 (after 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 [47].

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 [24,25]. 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. [25]. 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 [48].

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 two months and an increase of NO_3^-. Despite the decline, NH_4^+ remains significantly higher when compared to the controls even four months after the cadavers were placed. This groups pH additionally into LDMs, NH_4^+ additionally into LPMs and NO_3^- into LPMs. It suggests a return of aerobic conditions allowing aerobic nitrification after an initial lag phase [48,22]. This follows a pattern shown by Meyer et al. [15] 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 [27].

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. [25] 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 26 g kg^{-1} N concentration is reported for pigs [25]. 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 [29]. N was grouped into EPMs with continuing elevated levels up to almost one year. This can be confirmed by other studies that have shown that total N was significantly higher after one year beneath decomposing pigs [2,27]. Here more data will be necessary.

Although carbon accounts for 20% of the mass of cadavers [1] no significant changes were observed in the soil beneath the cadavers, which is in line with other studies [15,25,27]. 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 [29].

The input of P from cadavers, where P is stored in proteins, coenzymes, sugar phosphates and phospholipids [4], may translate into a large increase in soil as available P [34]. 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 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. [25] and Perrault and Forbes [34]. Additionally, [29] 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 [23]. Phosphorous concentration seems to be a good indicator for locating the decomposition of remains [34].

Potassium was also grouped into the EPMs and the early phase of the LPMs. Assuming that 100 g of pig body tissue contain approximately 280 mg K [49] 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 [24] and Stokes et al. [22] 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. Here more refined categories will be necessary and more data needs to be collected to achieve this goal. Above all more data is needed from real caseworks and studies with human bodies to develop a method that could be valid in court. A first attempt on how this method in combination with others could be useful in a real case investigation was presented recently by Szelecz et al. [50]. Using the marker categories it was shown that either the time elapsed since death was sufficient for EPMs to return to basal levels or the body had started decomposing elsewhere and was transported to the find site [50]. A In further studies, especially over longer periods of time i.e. several years more key elements should be investigated e.g. the skeletal components such as calcium and magnesium that are released in later stages of decay. This will also help to improve and define the markers more precisely. 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, especially when the changes in soil chemistry are related to changes in the composition of soil organism communities, 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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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.forsciint.2018.02.031.

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