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Soil volatile analysis by proton transfer reaction-time of flight mass spectrometry (PTR-TOF-MS)

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ABSTRACT

We analyzed the volatile organic compounds (VOCs) emitted from different soils by using the PTR-MS-TOF technique under laboratory conditions and compared them with soil chemical biochemical activities. The emitted VOCs were related to soil microbial biomass, soil respiration and some soil enzyme activities so as to evaluate if size and activity of soil microbial communities influenced the soil VOCs profiles. Our results showed that the emitted VOCs discriminated between soils with different properties and management, and differences in the VOCs emission profiles were likely related to the active metabolic pathways in the microbial communities of the three studied soil. Our results also showed that some soil enzyme activities such as β-glucosidase and arylsulfatase were possibly involved in the release of compounds fueling microbial metabolic pathways leading to the production of specific VOCs. It was concluded that the PTR-MS-TOF technique is suitable for analyze VOCs emission from soil and that studies comparing soil enzyme activities and soil volatile profiles can reveal the origin of VOCs and give further insights on microbial activity and soil functionality.

1. Introduction

Increasing concern on the global environmental change induced by increased emissions of greenhouse gases (GHG) such as CO_2, CH_4, N_2O has led climatologists, plant and soil scientists to determine the GHG fluxes from natural sources and sinks such as water bodies, plants and soils (Batjes et al., 1995; IPCC, 2007). Different groups of microorganisms can contribute to the GHG emissions from soil under various pedo-climatic conditions (Lu et al., 2012; Lau et al., 2013). However, the gaseous emission from soil include not only CO_2, CH_4, N_2O but also several volatile organic compounds (VOCs) released by the active metabolic pathways of soil microorganisms, fauna and plant roots (Isidorov and Jdanova, 2002; Asensio et al., 2007; Leff and Fierer, 2008; Ramirez et al., 2010; Gray et al., 2010; Seewald et al., 2010).

It has been estimated that VOCs emissions can account for 10–30% of the total soil emissions (Gray et al., 2010), and depend on soil physico-chemical properties, environmental conditions such as soil temperature and moisture levels (Schade and Custer, 2004; Asensio et al., 2007), with seasonal trends in the emissions (Aaltonen et al., 2013). VOCs emission profiles of soils also respond to changes in the vegetation cover (Kesselmeier and Staudt, 1999) and soil organic and inorganic fertilization (Gray and Fierer, 2012).

Soil emission of VOCs also depend on VOCs sorption by clay minerals and partition between the soil gas phase and soil solution (Isidorov and Jdanova, 2002; Asensio et al., 2007). An additional factor influencing the soil volatile analysis profile is VOCs degradation by abiotic reactions with hydroxyl and nitrate radicals (Atkinson and Arey, 2003) and degradation by soil microorganisms (Leff and Fierer, 2008; Insam and Seewald, 2010).

Soil VOCs emission can also have important influences on the ecosystem processes by stimulating or inhibiting plant growth (Hung et al., 2013). For example, several soil borne bacteria (e.g., *Pseudomonas* sp., *Bacillus* sp.) are known to produce fungistatic VOCs which can antagonize plant root infection, whereas other bacteria release VOCs with plant growth promoting effects (Wilkins, 1996; Kloepper et al., 2004). On the other hand, plants produce VOCs with microbial antagonistic effects (Dorman and Deans, 2000); some VOCs are also produced by plants as metabolic responses related to specific ecological interactions such as those occurring between plant host and parasite (Bitas et al., 2013; Fiers et al., 2013) or herbivory (Danner et al., 2012).

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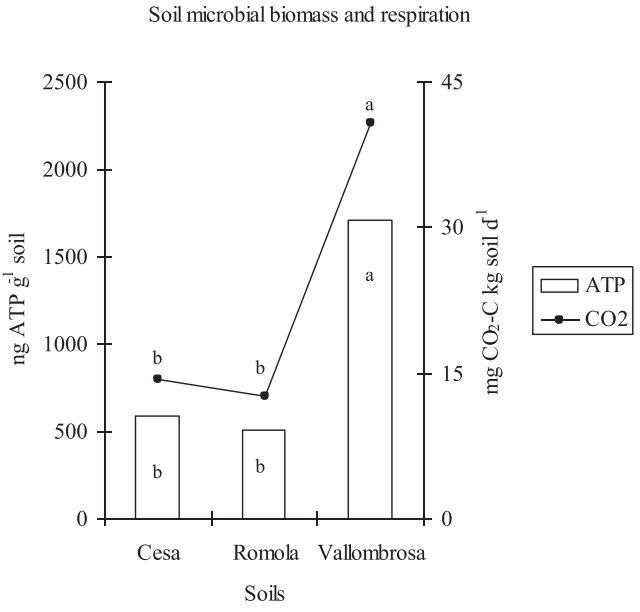

Fig. 1. Microbial biomass and respiration of Cesa, Romola and Vallombrosa soils. Different letters indicate significant differences ($P < 0.05$) among the mean values ($n = 5$).

A comprehensive assessment of VOCs emission may give further insights not only on the effects of soil type on their production but also on their potential role in soil functionality (Holopainen and Gershenzon, 2010). VOCs emission profile from soil can be assessed in laboratory and field experiments by various techniques including Fourier transform infra-red spectrometry (FTIR) (Puckrin et al., 1996), gas chromatography coupled with mass spectrometry (GC–MS) (Smet et al., 1999), and those based on proton transfer reaction (PTR) such as PTR-mass spectrometry (PTR-MS) (Warneke et al., 2003), PT ion trap-mass spectrometry (PIT-MS) and negative ion PT chemical ionization mass spectrometry (NI-PT-CIMS) (Veres et al., 2008). The proton-transfer-reaction time-of-flight (PTR-TOF) is an innovative VOCs detection method that combines a high resolution time-of-flight mass spectrometer (TOF-MS) with a PTR ion source. It is rapid and highly sensitive and for these reasons it has been used to monitor of VOCs evolved from plant tissues (Gray et al., 2010; Brilli et al., 2011; Soukoulis et al., 2013), microbial liquid cultures (Bunge et al., 2008) and soil samples (Danner et al., 2012).

According to Insam (2013), monitoring of VOCs emission from soil is an approach which can give insights on the soil functionality avoiding the drawbacks of other techniques based on soil extractions. VOCs emission profile from soil depends on the metabolic pathways active within soil microbial communities but to date research on the relation between VOCs emission profile and these metabolic pathways is not known. Moreover, while accurate assays have been set up for several soil enzymatic reactions involved in C, N, P and S cycles, many other enzyme reactions involved in soil microbial metabolism such as the intracellular enzyme activities involved in secondary metabolite synthesis and degradation can not be determined in soil because the related assays have not been set up (Nannipieri et al., 2012). Soil enzyme activities show distinctive patterns in different soils because it responds to main soil properties such as organic matter content, pH value, plant community and soil management (Renella et al., 2006); therefore the analysis of soil enzyme activity can be used to discriminate between soil types.

We hypothesized that VOCs profile of different soils can discriminate soils with different properties and metabolic activity, and provide indications on the main metabolic pathways active in the soil microbial communities by using the PTR-MS-TOF technique under laboratory conditions. The VOCs profiles were related to soil microbial biomass, soil respiration and soil enzyme activities so as to evaluate if size and activity of soil microbial communities influenced VOCs profiles. The determined enzyme activities were selected those mainly involved in C, N, P and S mineralization in soil and acquisition be soil microorganisms (Nannipieri et al., 2012), and in previous studies (Renella et al., 2006, 2007a) it was found that they showed characteristic trends in these three soils.

2. Materials and methods

2.1. Soil sampling and handling

An agricultural soil (Cesa), classified as clay loam Eutric Cambisol (WRB, 1998), a sandy-clay soil under mixed forest (Romola), classified as Eutric Cambisol (WRB, 1998) and a forest soil classified as sandy loam Fragic Disdrudept (WRB, 1998) located at Vallombrosa were sampled in June 2013. Six soil samples were collected from the A_P (0–30 cm) and A_0 (0–10 cm) horizons for the agricultural and forest soils, respectively, by using a stainless steel spade. For each sampling point, 4 sub-samples of ca. 0.5 kg were taken and mixed to obtain a composite sample. The six composite samples (ca. 2 kg) for each soil were kept separated before the analysis. The main soil chemical and physical parameters are reported in Table 1. Soils were sieved (<2 mm) at field moisture, moistened to 40% WHC and pre-incubated at 25 °C in the dark for 7 days to stabilize the soil microbial and biochemical activity after sampling, sieving and re-moistening. A schematic representation of the incubation conditions and headspace sampling is depicted in Fig. 1.

2.2. Soil volatile profile

Soil samples equivalent to 2 g dry weight were placed in glass Petri plates with exposed surface of 20 cm^2. The soil incubation and VOC sampling and analyses were performed in air conditioned rooms with a constant temperature of 25 ± 1 °C. The used setup and incubation conditions allowed the formation of a dynamic headspace sampling system with a constant the air flow of 0.3 L min^{-1} and a constant humidity, which are critical parameters in for VOCs determination (Warneke et al., 2001). Headspace sampling was performed 5 min after inserting the soil samples into the air-tight jars, which allowed to standardize measurements between soil samples meanwhile preventing the buildup of water

Table 1
Main physico-chemical properties, microbial biomass and respiration of the Cesa, Romola and Vallombrosa soils. Values with different superscript in each column are significantly different ($P < 0.05$).

	Sand	Silt	Clay	$pH_{(H_2O)}$	Organic C	Total N	NH_4^+-N	NO_3^--N	Total P	Available P
	(%)			(g kg^{-1})						
Cesa	32.1	42.2	25.7	7.8	10.8^b	1.172^b	0.62^b	0.081^b	129.1^b	6.45^b
Romola	81.9	6.7	11.4	6.9	6.97^c	0.081^c	0.026^c	0.027^c	63.6^c	8.85^a
Vallombrosa	77.8	20	2.2	4.8	29.9^a	2.693^a	0.92^a	0.151^a	179.0^a	7.39^b

vapor pressure and anoxic conditions. In fact, inside the air-tight jars the O_2 consumption in the headspace was in the order of 1% and the CO_2 concentration for the three soils ranged from 1000 to 2000 ppm after 24 h of incubation, which is same order of magnitude of the CO_2 concentration in the soil aggregates and unlikely inhibited the microbial activity in 5 min long incubations. After inserting the Petri plates containing the soil samples into 1 L air-tight jars, the three way valves were connected to two Teflon inlet tubes (3.2 mm diameter): one connected to the PRT-TOF-MS and the other connected to a zero-air generator (Peak Scientific, USA).

The VOCs protonation was carried out by using H_3O^+ as proton donor in the transfer reaction, and was effective for VOCs having a proton affinity higher than that of H_2O (691.7 kJ mol^{-1}). The dynamic headspace gas was then sampled with a capillary tube connected to a PTR-TOF-MS 8000 (Ionicon, Innsbruck, Austria). Detailed information on the PTR-TOF-MS instrumental set up are presented by Brilli et al. (2011). VOCs were measured by direct injection of the head space mixture into the PTR-TOF-MS drift tube via a hated (60 °C) peek inlet tube with a flow rate of 100 sccm. Measurements were carried out as previously illustrated by Cappellin et al. (2013) using a PTR-TOF-MS in its standard configuration. The sampling time for each channel of TOF acquisition was 0.1 ns, amounting to 350,000 channels for a mass spectrum ranging up to *m/z* 250 (Sanchez del Pulgar et al., 2013; Taiti et al., 2014). The conditions in the drift tube were: drift voltage 600 V, temperature 110 °C, pressure 2.25 mbar, extraction voltage at the end of the tube (Udx) 32 V. The PRT-TOF-MS was operated at an E/N value of 140 (1 Td = 10217 V cm^2).

The raw PTR-TOF-MS data are acquired by the TofDaq software (Tofwerk AG, Switzerland), using a dead time of 20 ns for the Poisson correction. In order to guarantee high mass accuracy throughout the analyses, compounds of exactly known *m/z* (1,4 dichlorobenzene, *m/z* = 146.976, and 1,2,3 trichlorobenzene, *m/z* = 180.937) were continuously added to the sample inlet system through a diffusive cell, and together with other known low mass ions (NO peak *m/z* = 29.9974 and $C_3H_7O^+$, *m/z* = 59.0497) these were used for internal calibration during data acquisition and post processing analyses for a precise conversion of time-of-flight into *m/z* values, in order to assign the exact mass and the sum formula of all ions during VOCs analysis (for more details see Brilli et al., 2011). The average signal intensity recorded for 60 s (300–360 s from the beginning of the acquisition) when the signal intensity reached constant values after the enrichment phase (Fig. S-1) was used for VOCs identification and data modeling and soils were characterized by their average mass spectra (in the range of *m/z* = 30–250), with a mass accuracy of 0.001 Th. Subsequently, we applied the empirical transmission curve to estimate the actual abundances of product ions over the product ions with mass range 30–250 and the peak intensities for the different VOCs were converted in ppbv using the formula described by Lindinger et al. (1998), and using constant values for the reaction rate coefficient ($kR = 2.10^{-9}$ cm^3 s^{-1}) to calculate concentration for each VOC (Fabris et al., 2010; Cappellin et al., 2012). For VOC identification and data modelling, we used an average signal intensity recorded for 60 s, which allowed the acquisition of 60 average spectra.

Before each experiment VOCs were determined in the headspace of empty glass jar identical to those used for soil measurements and kept in the same conditions for background subtraction, which was always close to the baseline (Fig. S-1). Given the aim of the present study the low noise present in the background (i.e., the blanks) and the very high sensitivity of the PTR-TOF-MS, there were no problems related with VOC detection.

VOCs identification was based on the fragmentation patterns of pure standards available in the literature (Sanchez del Pulgar et al., 2013; Soukoulis et al., 2013). We characterized each detected ion by its sum formula, complemented by tentative assignment of molecules based on the VOCs detected in previous on (see Table 3).

2.3. Soil chemical and biochemical analysis

Soil microbial biomass was determined by the ATP content according to Ciardi and Nannipieri (1990). Soil respiration was the CO_2-C emission rate determined by gas chromatography (HP 5890) using the same incubation conditions as for the VOCs determinations (Blackmer and Bremner, 1977). The arylesterase activity was determined as described by Zornoza et al. (2009). The acid and alkaline phosphomonoesterase activities were assayed according to Tabatabai and Bremner (1969), and the phosphodiesterase activity as reported by Browman and Tabatabai (1978). The urease activity was determined according to Nannipieri et al. (1974), the protease activity according to Ladd and Butler (1972), and the β-glucosidase activity according to Tabatabai (1982).

The total organic C (TOC) was determined by the method of Walkley and Black (1934). Inorganic N (NH_4^+-N and NO_3^--N) was extracted by shaking 5 g fresh soil for 1 h with 1 M KCl (1:5 soil: solution ratio) according to Keeney and Nelson (1982) and analyzed by ion selective electrodes. The available P was determined according to Olsen and Sommers (1982) for the alkaline (Cesa) and neutral (Romola) soils, and according to Bray and Kurtz (1945) for the Vallombrosa acidic soil.

2.4. Data analysis

A PLS-DA approach was used to characterize each soil according to chemical properties and VOCs, respectively. The PLS-DA consists of a classical Partial Least Squares (PLS) regression analysis where the response variable is categorical (y-block; replaced by a set of dummy variables describing the different soils), expressing the class membership of the statistical units (Sjöström et al., 1986; Sabatier et al., 2003 Menesatti et al., 2013). The model includes a calibration and a cross-validation phase. The x-blocks (chemical variables and VOCs) were pre-processed using a autoscale algorithm (i.e., centers columns to zero mean and scales to unit variance). A prediction probability of each modeled y-block categories (i.e., soil identity) was calculated for each sample according to Forina et al. (2008). Threshold values of the prediction probability were calculated for each y-block category using the observed distribution of predicted values and Bayesian statistics. By this way, an object could belong to none (outlier), one or more than one category, if the prediction probabilities for each category exceed the threshold values. This analysis also expressed the statistical parameters indicating the modeling efficiency in terms of sensitivity and specificity of parameters. The sensitivity was the percentage of samples of a category accepted by the class model. The entire dataset was subdivided into two groups: (1) 3 samples (one for each site) were used for the independent test (i.e., validation); (2) the rest were used for the class modeling and cross-validation. This partitioning was optimally chosen with the Euclidean distances calculated by the algorithm of Kennard and Stone (1969) by selecting parameters without a priori knowledge of a regression model. Each LV model with the mean higher performance value was considered to be the most robust one, according to Swierenga et al. (1998). Moreover, the Variable Importance in Projection (VIP) scores were calculated (Chong and Jun, 2005) and used to estimate the importance of each variable in predicting the soil identity according to the PLS-DA model. VOCs with VIP scores significantly higher than 1 were considered of major importance and might be good candidates for discriminating the soil identity. The models were developed by using a procedure written in the MATLAB 7.1 R14 software.

Table 2
Microbial biomass, basal respiration and enzyme activities of the Cesa, Romola and Vallombrosa soils. Values with different superscript in each column are significantly different ($P < 0.05$).

Soils	Microbial biomass	Basal respiration	Arylest.	Arylsulf.	Ac. phosph.	Alk. phosph.	Phosphod.	β-Gluc.	Urease	Protease
	(μg ATP kg^{-1} soil)	(mg CO_2-C kg^{-1} soil*d^{-1})	(mg *p–np* nkg^{-1} h^{-1})						(mg NH_4^+-N kg^{-1} h^{-1})	
Cesa	590.1^b	14.4^b	207.0^b	456.8^c	898.5^b	2355.2^b	1470.4^b	1907.1^a	140.4^a	113.9^a
Romola	505.4^b	12.7^b	99.8^c	1100.9^b	1247.3^b	4055.9^a	2169.0^a	943.6^b	73.6^b	117.7^a
Vallombrosa	1707.4^a	40.8^a	323.5^a	2524.7^a	10013.5^a	3721.4^a	1552.6^b	1872.9^a	45.3^b	85.8^b

Arylest. is the arylesterase actvitiy; Arylesulf. is the arylsulfatase actvitiy; Ac. phosph. is the acid phosphomonoesterase activity; Alk. phosph. is the alkaline phosphomonoesterase activity; Phosphod. is the phosphodiesterase activity; β-gluc. is the β-glucosidase activity.

3. Results and discussion

3.1. Soil chemical properties, biochemical activity and VOCs emission

The Cesa agricultural soil, Romola forest soil and Vallombrosa forest soil soils had different inorganic N (NH_4^+–N, NO_3^-–N) and available P concentrations (Table 1), with the Vallombrosa soil showing significantly higher values than the Cesa and Romola soils, except for the available P which was significantly higher in the Romola soil (Table 1). The microbial biomass and respiration values were significantly higher in the Vallombrosa soil than in the Cesa and Romola soils (Table 2). The three soils had also showed different soil enzyme activity, with the Vallombrosa soil showing significantly higher arylesterase, arylsulfatase and acid phosphomonosterase and significantly lower urease and protease activities than the Romola and Cesa soils (Table 2). It is well established that soil microbial biomass, microbial activity and enzyme activities are correlated to the soil organic matter content, with the enzyme activities also depending on soil pH value and soil management (Nannipieri et al., 2012). The arylesterase, arylsulfatase, acid phosphomonoesterase and β-glucosidase activities were significantly and positively correlated with microbial biomass, respiration, total organic C, N and P, and inorganic N (NH_4^+–N, NO_3^-–N), only the, β-glucosidase activity was negatively correlated with the available P, the phosphodiesterase showed significant negative correlations with total N and inorganic N and total P, and a positive correlation with the available P (Table 3). The protease activity showed negative significant correlations with microbial biomass, respiration total organic C and, with total N and inorganic N, whereas the urease showed negative significant correlations with only microbial biomass, respiration total organic C (Table 3); both urease and protease activities were not correlated to available P concentrations (Table 3). The three soils were well discriminated on the basis of the measured soil chemical properties and biochemical activities (Fig. 2A). Enzyme activity is generally correlated with microbial biomass, soil respiration and organic matter content (Frankenberger and Dick, 1983) and generally responds to the nutrient availability in soil (Allison and Vitousek, 2005; Renella et al., 2007b) although has been reported that such a correlation may vary depending on the soils and also in relation to soil management (Nannipieri et al., 2012).

3.2. Class-modeling analysis of the results

More than 200 peaks with a *m/z* ratio range of 30–250, derived from the protonation of various VOCs were detected from the three soils. VOCs with the highest VIP values were: isopentyl acetate (*m/z* = 131.1066) evolved from the Vallombrosa soil, hexenal (*m/z* = 81.0699) evolved from the Romola soil, and propanal or 2-propanone (*m/z* = 59.0491) evolved from the Cesa soil (Table 4). The protonated masses, molecular formula, and VIP scores of all the identified VOCs and related references are reported in Table 1. The three analyzed soils produced all complex PTR-TOF-MS spectra that resulted in large datasets (see Table S2) that required data mining approaches for their optimal exploitation (for further details see Müller et al., 2010, 2013; Holzinger et al., 2010; Taiti et al., 2014). In the present paper we used a class-modeling approach to analyze the data recorded by PTR-TOF-MS according, previously used by Taiti et al. (2014), and the lack of large variability in VOCs detection indicated that our assessment of soil VOC detection was sufficiently accurate (Table 5).

The performance indicators of models based on soil chemical, microbiological and biochemical properties and VOCs with 2 and 3 LVs, respectively, and selected as the more robust are reported in Table 4. Both models discriminated among all the three soils. The specificities (i.e., the percentage of samples of the categories different from the modeled one, which was rejected by the class model). and sensitivities (i.e., the percentage of the samples of a category accepted by the class model) were both 100%, the mean classification errors were equal to zero and RMSEC values were low (<0.4). The plot of scores of soils were grouped according to the soil type; the first two LVs represented both models based on soil chemical and biochemical properties and VOCs (Fig. 3). Fig. 3A

Table 3
Pearson correlation matrix of enzyme activities and microbial biomass, soil respiration and soil organic C contents of the Cesa, Romola and Vallombrosa soils. Symbols *, ** and *** indicate significant levels at $P < 0.05$, 0.01 and 0.001, respectively. Symbol NS indicate non significant correlations ($P > 0.05$).

	Arylest.	Arylsulf.	Ac. phosph.	Alk. phosph.	Phosphod.	β-Gluc.	Urease	Protease
	(mg *p–np* kg^{-1} h^{-1})						(mg NH_4^+-N kg^{-1} h^{-1})	
Microbial biomass (ng ATP kg^{-1})	0.815***	0.924***	0.960***	NS	NS	0.523*	−0.587*	−0.856***
Respiration (mg CO_2-C kg^{-1} soil d^{-1})	0.877***	0.921***	0.982***	NS	NS	0.593*	−0.573*	−0.882***
Organic C (g kg^{-1})	0.893***	0.901***	0.967***	NS	NS	0.543*	−0.545*	−0.872***
Total N (g kg^{-1})	0.948***	0.741***	0.863***	NS	−0.541*	0.747**	NS	−0.820***
Total P (g kg^{-1})	0.886***	0.602**	0.719**	NS	−0.661**	0.804***	NS	−0.685*
NH_4^+-N (g kg^{-1})	0.956***	0.496*	0.725**	NS	−0.862***	0.892***	NS	−0.718*
NO_3^--N (g kg^{-1})	0.920***	0.657*	0.823***	NS	−0.681**	0.782**	NS	−0.771**
Available P (g kg^{-1})	NS	NS	NS	0.670**	0.551*	−0.701**	NS	NS

Arylest. is the arylesterase actvitiy; Arylesulf. is the arylsulfatase actvitiy; Ac. phosph. is the acid phosphomonoesterase activity; Alk. phosph. is the alkaline phosphomonoesterase activity; Phosphod. is the phosphodiesterase activity; β-gluc. is the β-glucosidase activity.

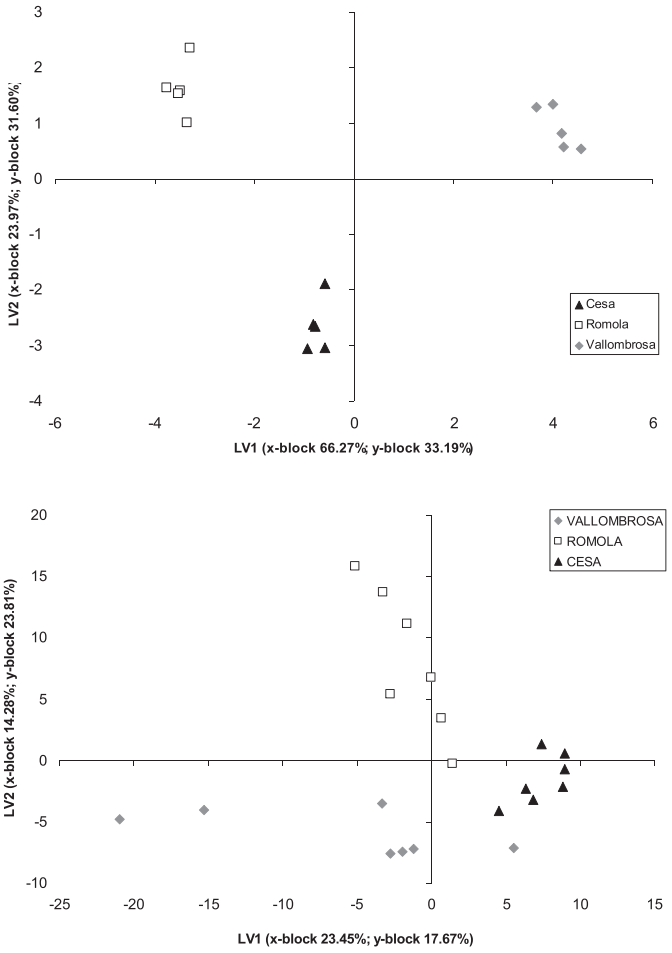

Fig. 2. Representation of the different soils on the first two axes of the PLS-DA (LV) models based on chemical, biochemical and microbiological properties (A) and VOCs (B) with 2 and 3 LVs, respectively.

represents the scores of the soils based on chemical and biochemical properties on the 2 axes (LVs) representing the whole model; the three groups are perfectly distinguishable. Fig. 2B represents the scores of the soils based on VOCs on the first two axes (LVs) representing the most of the explained variance (*x*-block 37.73%; *y*-block 41.48%) of the model (based on three LVs); the three groups are perfectly distinguishable only using all the three exes (LVs). In both cases a clear separation among soils was observed (Fig. 3). The threshold values calculated for chemical variables were 0.14 for the Cesa soils, 0.18 for the Romola soil and 0.29 for the Vallombrosa soil. The threshold values based on VOCs were 0.08 for Cesa soil, 0.02 for Romola soil and 0.13 for Vallombrosa soil. Considering the prediction probabilities of chemical properties dataset, all samples exceeding the threshold values only the soil type (i.e., they were properly modeled in the original class). Considering the prediction probabilities of VOCs dataset, all samples from Romola were correctly modeled; 4 out of 6 samples from Cesa soil were correctly modeled while the other 2 (one of them is the test observation) were modeled as Vallombrosa; 4 out of 6 samples from Vallobrosa soil were correctly modeled while 2 (both in the training set) were modeled either as Vallombrosa or Cesa soil.

3.3. Analysis of the VOCs emission profile of the studied soils

According to the data modelling, specific VOCs characterized the three soils (Table 1). In particular, the Vallombrosa soil was discriminated by methanol, ethanol and isoprene emissions, the Romola soil was discriminated by cyclopentane, methoxybenzene, santene, isopentyl acetate and monoterpenes emissions, whereas the Cesa soil was discriminated by the emission of eight VOCs (Table 3). With the exception of the formaldehyde, mainly emitted from the Cesa soil, VOCs characterizing the Vallombrosa soil had the smallest *m*/*z* ratio values (33.033–69.0698), the Romola soil was discriminated by VOCs with the highest *m*/*z* ratio values (81.069–137.134), whereas the Cesa soil was discriminated by VOCs with the broadest *m*/*z* ratio values (31.0191–127.108). Possibly, the chemical properties and biological activity of the three soils influenced not only the biochemical activities but also VOCs emission profile.

The Cesa soil showed the highest rates of formaldehyde and acetaldehyde production and the lowest rates of methanol and ethanol emission (Table 1). Formaldehyde is produced by all microrganisms, particularly by methylotrophic bacteria, as a result of demethylation reactions (Vorholt et al., 2000), and it undergoes to fast intracellular turnover rates. Probably the activity of methanotrophic and methylotrophic microorganisms was higher in the Cesa soil than from the Romola and Vallombrosa soils. Differently, the Vallombrosa soil showed the highest rates of methanol emission and the lowest rates of formaldehyde production, whereas the Romola soil showed intermediate rates for the two VOCs (Table 1). Methanol in soil is produced during the microbial breakdown of plant polysaccharides rich in galacturonic acid (e.g., pectin), where the carboxylic acids are mostly esterified with methanol (Micheli, 2001). The opposite trends of formaldehyde and methanol emission from the three soils let us to hypothesize that different groups of microorganisms and metabolic pathways were active in the three soils. From the obtained results it is likely that more methylotrophic bacteria were active in the Cesa soil than in the Vallombrosa soil whereas in the Romola soil the two metabolic pathways were equally active (Fig. 3).

Ethanol and acetaldehyde emission from soil could also indicate the presence in soil of auxin precursors such as indole-3-ethanol and indole-3-acetaldehyde (Frankenberger and Brunner, 1983; Brown and Hamilton, 1992). The conversion of indole-3-acetaldehyde to indole-3-ethanol acid depends on microbial respiration and is mediated by specific oxidases synthesized by soil borne bacteria (Uchida et al., 2003).

The 2-propanone/propanal (acetone) emission was higher from the Cesa soil than from the Romola and Vallombrosa soils, which paralleled the high emission of formaldehyde and acetaldehyde from the former soil, and indicating that not all the produced 2-propanone/propanal2-propanone/propanal was converted to aldehydes by soil microorganisms (Vestal and Perry, 1969). Emission of 2-propanone/propanal from soil has been already reported (Schade and Custer, 2004); VOCs such as 2-propanone/propanal and acetaldehyde can be formed by chemical reactions between sugars and proteins not necessarily catalyzed by enzymes (i.e., Maillard reaction) during the SOM decomposition (Warneke et al., 1999). Moreover, alcohols and carbonylic compounds, like other VOCs, are chemically reactive and their emission from different soils may depend on both the release from active microbial metabolic pathways and on their sorption and/or reaction with surface reactive soil colloids. The highest rates of acetate and 2-propanone/propanal emission from the Cesa soil may suggest that in this soil 2-propanone/propanal was also oxidized to acetate and CO_2 by soil microorganisms (Taylor et al., 1980).

The significantly higher isoprene emission from the Vallombrosa than from the Romola and Cesa soils may be related to the significantly higher soil microbial biomass and nutrient availability of the Vallombrosa soil as isoprene is a metabolite produced by soil microorganisms (Sivy et al., 2002). Although

Table 4
Molecular mass, mass/charge (m/z) ratios and identified of VOCs evolved from Vallombrosa, Romola and Cesa soils. Values are means ± stdev. Values of VIP scores in bold indicate the discriminant compounds ($P = 0.01$) among the three soils.

Protonated measured (m/z)	Protonated chemical formula	Protonated theoretical (m/z)	Tentative identification	VOCs concentration (ppbv)			VIP scores			References for PTR-MS-TOF	References for soil studies
				Vallombrosa	Romola	Cesa	Vallombrosa	Romola	Cesa		
31.0191	CH_3O^+	31.0178	Formaldehyde	0.30 ± 0.06	0.85 ± 0.11	1.60 ± 0.30	1.002	1.417	**1.660**		Asensio et al., 2007
33.033	CH_5O^+	33.0335	Methanol	10.48 ± 1.51	2.50 ± 0.36	1.85 ± 0.29	**1.393**	1.211	1.148	Sanchez del Pulgar et al., 2013	Seewald et al., 2010
45.033	$C_2H_5O^+$	45.0334	Acetaldheyde	10.58 ± 0.68	12.00 ± 0.41	4.13 ± 0.85	1.275	1.115	**1.763**	Brilli et al., 2011	Bunge et al., 2008
47.048	$C_2H_7O^+$	47.0491	Ethanol	0.95 ± 0.29	1.87 ± 0.30	4.29 ± 1.12	**1.973**	1.941	1.137	Galle et al., 2001	Asensio et al., 2007; Seewald et al., 2010
59.048	$C_3H_7O^+$	59.0491	2-Propanone/ propanal	7.16 ± 0.72	5.23 ± 0.44	3.93 ± 0.40	1.294	1.652	**2.372**	Galle et al., 2001	Seewald et al., 2010
61.028	$C_2H_5O_2^+$	61.0284	Acetic acid/ acetate	9.84 ± 0.80	11.35 ± 1.00	2.95 ± 0.80	1.262	0.713	**1.507**	Bunge et al., 2008; Galle et al., 2001	Seewald et al., 2010
63.027	$C_2H_7S^+$	63.0268	Dimethylsulfide (DMS)	0.70 ± 0.10	0.70 ± 0.20	0.20 ± 0.05	1.199	1.231	**1.788**	Galle et al., 2001	Morath et al., 2012
69.067	$C_5H_9^+$	69.0698	Isoprene	1.25 ± 0.20	1.70 ± 0.25	0.80 ± 0.04	**1.674**	1.276	1.225	Galle et al., 2001	Mayrhofer et al., 2006; Asensio et al., 2007
81.069	$C_6H_9^+$	81.0699	Hexenal	0.40 ± 0.10	1.80 ± 0.21	0.50 ± 0.10	1.750	**2.270**	1.449	Brilli et al., 2011	Isidorov and Jdanova 2002
83.050	$C_5H_7O^+$	83.0491	Methylfuran	0.57 ± 0.07	0.30 ± 0.07	0.20 ± 0.02	1.187	0.445	**1.300**	Özdestan et al., 2012	Isidorov and Jdanova, 2002
85.065	$C_5H_9O^+$	85.0647	Cyclopentanone	1.45 ± 0.49	4.30 ± 0.43	3.25 ± 0.32	1.393	**1.529**	0.831		Wilkins et al., 2000
91.060	$C_4H_{11}S^+$	91.0575	Isopropyl methyl sulfide	0.29 ± 0.03	0.35 ± 0.05	0.15 ± 0.04	1.327	0.899	**1.543**		Isidorov and Jdanova, 2002
109.076	$C_7H_9O^+$	109.0647	Methoxybenzene/	0.20 ± 0.06	0.70 ± 0.10	0.50 ± 0.05	1.353	**1.563**	0.707		Isidorov and Jdanova 2002; Leff and Fierer, 2008
123.115	$C_9H_{15}^+$	123.1168	Santene	0.39 ± 0.05	0.75 ± 0.10	0.46 ± 0.03	1.666	**1.844**	0.535		Isidorov et al., 2003
127.108	$C_8H_{15}O^+$	127.1117	1-Octen-3-one/6-methyl-5-hepten-2-one	0.10 ± 0.03	0.40 ± 0.04	0.40 ± 0.02	1.208	1.275	**1.512**	Sanchez del Pulgar et al., 2013	Isidorov and Jdanova, 2002
131.101	$C_7H_{15}O_2^+$	131.1066	Isopentyl acetate	4.95 ± 0.66	10.10 ± 1.04	3.62 ± 0.30	1.848	**2.255**	1.201	Heenan et al., 2012	Isidorov and Jdanova, 2002; Asensio et al., 2007
137.134	$C_{10}H_{17}^+$	137.1325	Monoterpenes	0.44 ± 0.10	1.68 ± 0.10	0.41 ± 0.05	1.579	**2.075**	1.363	Galle et al., 2001	Isidorov and Jdanova, 2002

some of the enzymes involved in the isoprene synthesis and degradation have been detected, the physiological role of isoprene in microbial cells is not yet clarified (Hess et al., 2013).

Unsaturated alcohols, aldehydes and ketones such as 6-methyl-5-hepten-2-one (sulcatone) and 1-Octen-3-one (amyl vinyl ketone), are metabolic intermediates in several soil microorganisms including bacteria, cyanobacteria and fungi; they can be synthesized as intermediate metabolites of the terpene and chetoester degradative pathways in plants and microorganisms (Peters et al., 1992; Fruekilde et al., 1998; Höckelmann et al., 2004; Culler et al., 2010). These compounds may play important roles in the ecological interactions among soil microorganisms. For example, 1-octen-3-ol or mono- and sesquiterpenes are fungistatic VOCs, produced by actinobacteria and by the bacterial genera of *Bacilllus* and *Pseudomonas*, and emission of these compounds from soils colonized by plant roots has been found to play important roles in ecological interactions between plants and herbivorous insects (Weissteiner et al., 2012).

Santene (dimethylbicyclo[2.2.1]hept-2-ene), emission showed the highest rates from the Romola soil (Table 1). Santene belongs to a class of bicyclic compounds produced by plants and microorganisms and can be a precursor of phytohormones (e.g.,

Table 5
Results of the PLS-DA models based on soil chemical, biochemical and microbiological properties and VOCs evolved from Vallombrosa, Romola and Cesa soils.

Model variables	Soil chemical parameters	VOCs
N	15	21
n° units (*Y*-block)	3	3
n° LV	2	3
% Cumulated variance *X*-block	90.24	48.33
% Cumulated variance *Y*-block	64.79	54.98
Mean specificity (%)	100.00	100.00
Mean sensitivity (%)	100.0	100.0
Random probability (%)	33.33	33.33
Mean class. err. (%)	0.00	0.00
Mean RMSEC	0.34	0.39
% Corr. class. model	100	100
% Corr. class. independent test	100	100

N is the number of samples; n° units (*Y*-Block) is the number of species to be discriminated by the PLSDA; n° LV is the number of latent vectors for each model; random probability (%) is the probability of random assignment of an individual compound into a unit.

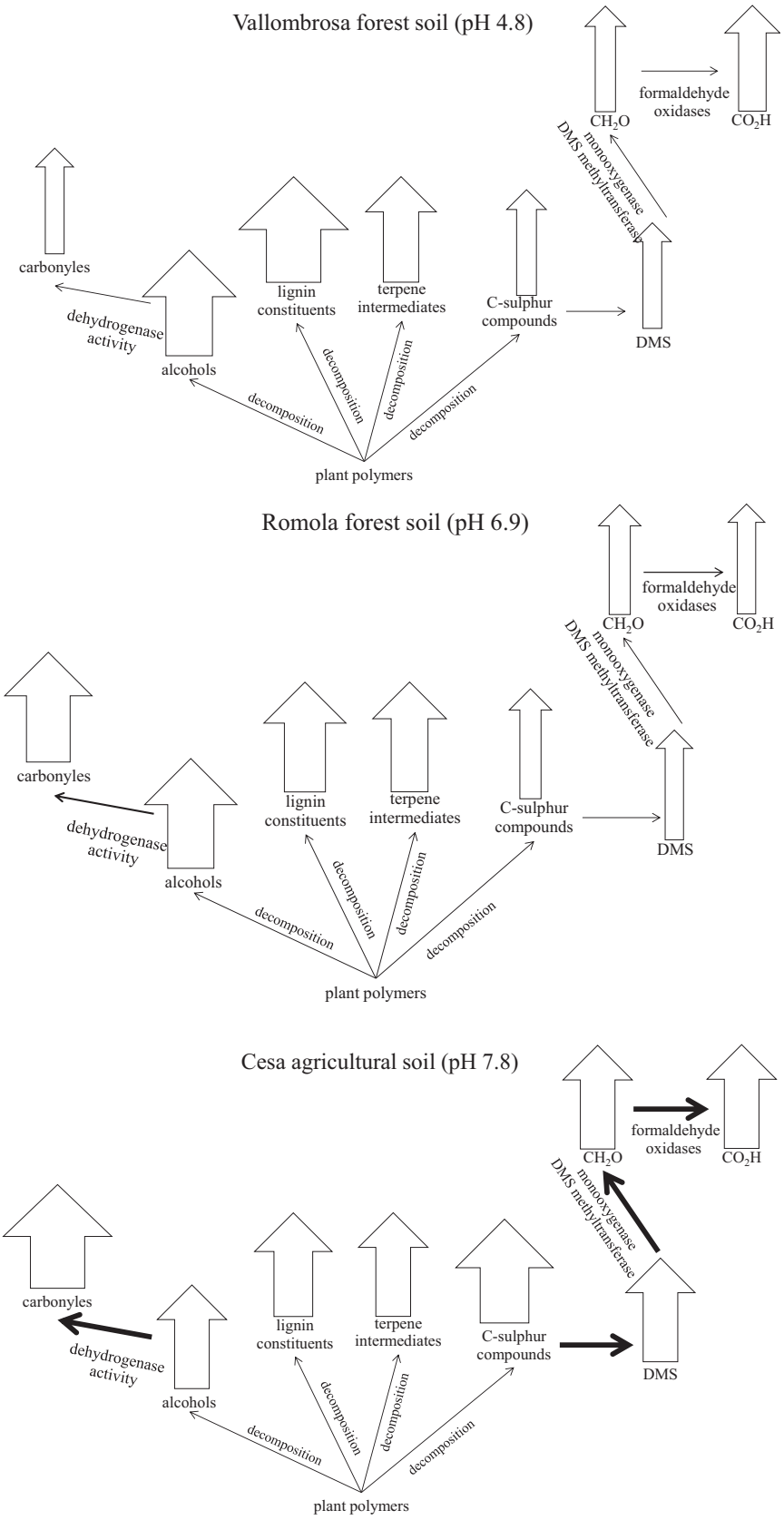

Fig. 3. Different metabolic processes in Vallombrosa, Romola and Cesa soils based on the VOCs emissions. The width of the arrows represents the rate of the metabolic processes.

gibberellin) monoterpenes or alkaloids. In general, monoterpenes and isopentil-derivatives, which characterized the VOCs emissions from the Vallombrosa and Romola soils (Table 2), are also precursors of gibberellins and cytokinins synthesized by soil rhizobacteria (Arshad and Frankenberger, 1991). Stimulation of microbial activity and enzyme activity in soils by trace amounts of phytohormone precursors has been reported (Renella et al., 2011a), and isopentenyl-adenosine with cytokinin-like activity has been detected in humic substances and earthworm faeces (Pizzighello et al., 2013).

Anisole (methoxybenzene) is a phenolic-ether and it is a component of plant polymers (e.g., lignin) and is emitted by plant roots (Weissteiner et al., 2012); it can be metabolized by soil microorganisms through demethylation or transformed to phenol and to various hydroxylated products (e.g., catechol) by dioxygenases (Resnick and Gibson, 1993). The high anisole emission from the Vallombrosa and Romola forest soils as compared to the Cesa soil may reflect the higher lignin accumulation in these two forest soils than in the Cesa agricultural soil.

Hexanal emission was higher from the Romola soil than from Vallombrosa and Cesa soils (Table 1). Aldehydes such as hexanal, heptanal and nonanal can be produced through the degradation pathways of unsaturated fatty acids during microbial growth (Korpi et al., 1998).

Dimethyl sulfide (DMS), an important intermediate molecule in the C and S biogeochemical cycles, is produced by microbial catabolism of sulfurilated compounds (Schäfer et al., 2010). While the metabolic pathways of DMS production in different bacteria have been elucidated (Todd et al., 2007), its production by soil microorganisms is poorly understood. Interestingly, the DMS emission was the highest in the Cesa soil, which also displayed the highest rate of isopropyl methyl sulfide production (Table 2). Probably, several methylsulfides and methanethiols may be precursors or intermediate compounds of the DMS metabolic pathway (Schäfer et al., 2010). The highest rates of emission of DMS, formaldehyde and acetate from the Cesa soil may also indicate the conversion of DMS to formaldehyde by microbial DMS monooxygenase/DMS methyltransferase and the possible conversion of formaldehyde to acetate by formaldehyde oxidases (Fig. 3). Moreover, the lowest isoprene production in the Cesa soil coupled with the highest acetate and DMS emission from this soil (Table 1), confirmed the evidences from in vitro studies that bacterial isoprene synthesis is inhibited by acetate and DMS (Hess et al., 2013). The fact that the Cesa soil had the lowest arylsulfatase activity among the studied soils could indicate that this activity may not be the directly involved in the S acquisition and metabolism of sulfurilated VOCs by soil microorganisms. The used sulfatase assay is specific to arylsulfate esters, but it can not be excluded that other S-esters may be hydrolyzed and the corresponding residual alcohol be volatilized.

In addition to differences in the vegetation cover, an important discriminating factor between the Vallombrosa and Romola soil is the acidic pH value of the former soil. Acidity can reduce the activity of microbial periplasmic dehydrogenases responsible for the conversion of C-1 and C-2 alcohols to aldehydes (Table 2), whereas the neutral pH value of the Romola soil may allow the activity of different microbial metabolic pathways.

It is important to underline that our PTR-TOF-MS approach enabled the analysis of the different soil VOCs emission profiles, although the precise assignment of the identified molecular masses to specific VOCs should be confirmed by coupling the PTR-TOF-MS with other analytical techniques (e.g., gas chromatography). The tentative assignment in this study was based on the on previous knowledge of the VOCs emitted by various soils (Table 3), and on the available knowledge on the secondary volatile metabolite emitted by soil microorganisms. Quantification of VOC emission rates from reactive heterogeneous environmental matrices such as soils need the assessment of several physico-chemical parameters (e.g., dissolved organic matter, active carbonate content), which must be all known for the selected VOCs and studied soils. To date, information on VOCs partition between the physical phases (i.e., solid particles, soil solution and gas phases) of different soils is still very limited and needs to be experimentally derived by laboratory future work based on incubation experiments, to appropriately calculate the VOCs emission rates.

Cesa and Vallombrosa soils showed the highest β-glucosidase activity and also the highest emission of ethanol, acetaldehyde and acetate (Tables 2 and 4), indicating that the glucose released by this enzyme activity contributed to the release of these VOCs, and probably to the microbial activity of these soils. The arylesterase activity was significantly higher in the Vallombrosa than in the Cesa and Romola soils probably due to the high availability of various alkyl and aryl esters in this forest soil (e.g., methoxy phenyl acetate, methyl- acetate, ethyl acetate). It is reasonable to hypothesize that the arylesterase activity could also be involved in the alcoholic VOCs emission from the Vallombrosa soil (Tables 2 and 4). The arylesterase activity in soil is more affected by the SOM than by other soil chemical or microbiological properties (Zornoza et al., 2009; Renella et al., 2011b). However, the influence of the enzyme activity on VOCs emission from soil need further investigation.

4. Conclusions

Our results showed that the volatile analysis profile can discriminate between soils with different properties and management. VOCs emission profiles suggest differences in the activity of soil microbial groups with different active metabolic pathways. Future research is needed to study how the composition of the soil microbial communities and the availability of organic substrates affect VOCs emission. Our results also showed that some soil enzyme activities like β-glucosidase and arylesterase activities were probably involved in the release of substrates for metabolic pathways leading to the release of specific VOCs. Studies comparing soil enzyme activities limiting the rate of metabolic pathways producing specific VOCs and soil volatile analysis profiles can reveal not only the origin of VOCs but also give further insights on microbial activity and soil functionality. An important aspect that deserve more research in the future is the exact quantification of VOCs emission rates from soil, as quantitative analysis of VOCs emission rates from soils will need the determination of coefficients of VOCs air/liquid/solid phase partition for different soils. Moreover, interpretation of the soil VOCs profile may greatly benefit from joint metagenomics and proteomic soil analysis to relate the VOCs detection to the active metabolic pathways in the soil microbial communities.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2014.10.018.

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