



Chemotaxonomic patterns of vegetation and soils along altitudinal transects of the Bale Mountains, Ethiopia, and implications for paleovegetation reconstructions – Part II: lignin-derived phenols and leaf-wax-derived *n*-alkanes

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Relevant dates: Received: 9 August 2018 – Revised: 6 March 2019 – Accepted: 25 July 2019 – Published: 4 September 2019

How to cite: Lemma, B., Mekonnen, B., Glaser, B., Zech, W., Nemomissa, S., Bekele, T., Bittner, L., and Zech, M.: Chemotaxonomic patterns of vegetation and soils along altitudinal transects of the Bale Mountains, Ethiopia, and implications for paleovegetation reconstructions – Part II: lignin-derived phenols and leaf-wax-derived *n*-alkanes, E&G Quaternary Sci. J., 68, 189–200, <https://doi.org/10.5194/egqsj-68-189-2019>, 2019.

Abstract: *Erica* is a dominant vegetation type in many sub-afroalpine ecosystems, such as the Bale Mountains in Ethiopia. However, the past extent of *Erica* is not well known and climate versus anthropogenic influence on altitudinal shifts are difficult to assign unambiguously, especially during the Holocene. The main objective of the present study is to chemotaxonomically characterize the dominant plant species occurring in the Bale Mountains using lignin phenols and *n*-alkane biomarkers and to examine the potential of those biomarkers for reconstructing vegetation history. Fresh plant material, organic layer and mineral topsoil samples were collected along a northeastern and a southwestern altitudinal transect (4134–3870 and 4377–2550 m a.s.l., respectively). Lignin-derived vanillyl, syringyl and cinnamyl phenols were analyzed using the cupric oxide oxidation method. Leaf-wax-derived *n*-alkanes were extracted and purified using Soxhlet and aminopropyl columns. Individual lignin phenols and *n*-alkanes were separated by gas-chromatography and detected by mass spectrometry and flame ionization detection, respectively.

We found that the relative contributions of vanillyl, syringyl and cinnamyl phenols allow us to chemotaxonomically distinguish contemporary plant species of the Bale Mountains. *Erica* in particular is characterized by relatively high cinnamyl contributions of > 40%. However, litter degradation strongly decreases the lignin phenol concentrations and completely changes the lignin phenol pat-

terns. Relative cinnamyl contributions in soils under *Erica* were < 40 %, while soils that developed under Poaceae (*Festuca abyssinica*) exhibited relative cinnamyl contributions of > 40 %.

Similarly, long-chain *n*-alkanes extracted from the leaf waxes allowed for differentiation between *Erica* versus *Festuca abyssinica* and *Alchemilla*, based on lower C_{31} / C_{29} ratios in *Erica*. However, this characteristic plant pattern was also lost due to degradation in the respective O layers and A_h horizons. In conclusion, although in modern-day plant samples a chemotaxonomic differentiation is possible, soil degradation processes seem to render the proxies unusable for the reconstruction of the past extent of *Erica* on the Sanetti Plateau, Bale Mountains, Ethiopia. This finding is of high relevance beyond our case study.

Kurzfassung:

Erica prägt als dominante Pflanzengattung viele Subafro-alpine Ökosysteme, so auch die Bale Berge in Äthiopien. Das Ausmaß der flächenhaften Ausdehnung von *Erica* in der Vergangenheit ist jedoch unklar, genauso wie die klimatische versus menschliche Verursachung solcher Vegetationsänderungen insbesondere im Holozän. Das Ziel dieser Studie war es herauszufinden (i) ob sich die dominante Vegetation in den Bale Bergen anhand von ligninbürtigen Phenolen und blattwachsbürtigen *n*-Alkanbiomarkern chemotaxonomisch unterscheiden lässt und (ii) ob diese Biomarker das Potential haben zur Vegetationsrekonstruktion im Untersuchungsgebiet beizutragen. In einem Begleitartikel (Mekonnen et al., 2019) verfolgten wir dasselbe Ziel, jedoch anhand von Stabilkohlenstoff- und Stickstoffisotopen sowie Zuckerbiomarkern. Untersucht wurden Pflanzenproben, O-Lagen und A_h Horizonte entlang eines Nord- und eines Südwest-Höhentransektes (3870–4134 m bzw. 2550–4377 m ü. NN). Die ligninbürtigen Phenoleinheiten Vanillyl, Syringyl und Cinnamyl wurden mittels der Kupferoxidationsmethode gewonnen; die *n*-Alkane wurden mittels Soxhlet extrahiert und über Aminopropylsäulen aufgereinigt. Die Quantifizierung der Ligninphenole und *n*-Alkane erfolgte mittels Gaschromatographie – Massenspektrometrie bzw. Gaschromatographie – Flammenionisationsdetektion.

Die Ergebnisse zeigen, dass sich die dominanten Pflanzenarten in den Bale Bergen anhand ihrer Vanillyl, Syringyl und Cinnamyl Einheiten chemotaxonomisch unterscheiden lassen. So weist insbesondere *Erica* charakteristischerweise relativ hohe Cinnamyl-Anteile von > 40 % auf. Vermutlich degradationsbedingt nimmt jedoch in der Reihe Pflanze – O-Lage – A_h Horizont nicht nur die Ligninkonzentration stark ab, sondern auch die Ligninmuster ändern sich völlig. Dadurch weisen Böden unter *Erica* Cinnamyl-Anteile < 40 % auf, während Böden die sich unter der dominanten Grasart *Festuca abyssinica* entwickelt haben Cinnamyl-Anteile von > 40 % aufweisen. Auch anhand der Alkanbiomarker ist eine chemotaxonomische Unterscheidung zumindest zwischen *Erica* versus *Festuca abyssinica* und *Alchemilla* möglich. Als Proxy dient hier das Verhältnis von C_{31} zu C_{29} . Allerdings führt auch hier Degradation in der Reihe Pflanze – O-Lage – A_h Horizont zum Verlust des charakteristischen Alkanmusters. Obwohl sich rezentes Pflanzenmaterial chemotaxonomisch unterscheiden lässt, zwingt dies zur Schlussfolgerung, dass Degradationseffekte bei der Rekonstruktion von *Erica* im Untersuchungsgebiet der Bale Berge in Äthiopien anhand von ligninbürtigen Phenolen und blattwachsbürtigen Alkanbiomarkern nicht unberücksichtigt bleiben dürfen. Dieser Befund ist über unsere Fallstudie hinaus von hoher Relevanz.

1 Introduction

The Bale Mountains are an eastern afro-montane biodiversity hotspot area with 27 endemic species of flowering plants (Hillman, 1988). Like in many other afro-montane ecosystems, an altitudinal zonation of the vegetation is well established, with an Ericaceous belt forming a prominent feature. Ericaceous vegetation dominates above 3300 m a.s.l., shows different stages of post-fire succession and remains continuous up to 3800 m a.s.l. However, it becomes patchy on the Sanetti Plateau (Miehe and Miehe, 1994). The Bale Moun-

tains National Park is increasingly under threat from climate change and anthropogenic impacts (Kidane et al., 2012). Ascertaining the past environmental and vegetation history of the area will support conservation efforts and may help to disentangle the influence of climate versus human impact on the present biodiversity.

Until now, the vegetation history of the Bale Mountains was studied using pollen records from lacustrine sediments and peat deposits (Bonnefille and Hamilton, 1986; Bonnefille and Mohammed, 1994; Hamilton, 1982; Umer et al., 2007). The results suggest the extension of the Ericaceous belt to-

wards higher altitudes during the early and middle Holocene. As potential drawbacks, such pollen studies depend on pollen preservation and can be biased by variable pollination rates as well as middle- and long-distance pollen transport (Hicks, 2006; Jansen et al., 2010; Ortu et al., 2006). By contrast, stable isotopes and biomarkers can also be applied to more degraded sedimentary archives and soils and are assumed to reflect the standing vegetation more (Glaser and Zech, 2005). Thus, they offer the potential to complement pollen-based vegetation reconstructions and to reconstruct vegetation at a higher temporal and spatial resolution. For instance, the stable carbon isotopic composition ($\delta^{13}\text{C}$) of lacustrine sediments suggests an expansion of alpine C_4 grasses on Mount Kenya, especially during glacial times (Street-Perrott et al., 2004), whereas $\delta^{13}\text{C}$ results from (paleo-)soils provide no evidence for C_4 grass expansion close to Mount Kilimanjaro during late Pleistocene glacial period (Zech, 2006; Zech et al., 2011b). We focus here on lignin-derived phenols and leaf-wax-derived *n*-alkanes as biomarkers, while stable isotopes and sugar biomarkers and their chemotaxonomic potential for reconstructions of the Bale Mountains vegetation are addressed in a companion paper by Mekonnen et al. (2019).

Lignin has a polyphenolic biochemical structure produced by terrestrial vascular plants (Ertel and Hedges, 1984) providing strength and rigidity to the plants (Thevenot et al., 2010). The lignin-derived phenols vanillyl (V), syringyl (S) and cinnamyl (C) as products of cupric oxide (CuO) oxidation are used to differentiate sources of organic matter and provide information about the diagenetic state (degree of degradation) of vascular plant material in terrestrial and aquatic sediments (Castañeda et al., 2009; Hedges et al., 1988; Tareq et al., 2004, 2006; Ziegler et al., 1986). For instance, low ratios of $\text{S}/\text{V} \sim 0$ were suggested as a proxy for the relative contribution of gymnosperms, and elevated S/V ratios were found to be indicative for the presence of angiosperms (Tareq et al., 2004). Likewise, the C/V ratio was proposed to indicate the relative contribution of woody ($\text{C}/\text{V} < 0.1$) and non-woody ($\text{C}/\text{V} > 0.1$) plants to the soil and sediment organic matter (Tareq et al., 2011). Moreover, the ratios of acid to aldehyde forms of vanillyl and syringyl units (Ac/Al)_{v,s} were suggested as proxies for quantifying the degree of lignin degradation (Amelung et al., 2002; Hedges and Ertel, 1982; Möller et al., 2002).

n-alkanes are important constituents of plant leaf waxes (Kolattukudy, 1970), where they serve to protect plants against water loss by evaporation as well as from fungal and insect attacks (Eglinton and Hamilton, 1967; Koch et al., 2009). Due to their recalcitrant nature, they are often well preserved in sedimentary archives and used as biomarkers (also called molecular fossils) in paleoclimate and environmental studies (Eglinton and Eglinton, 2008; Glaser and Zech, 2005; Zech et al., 2011c). The potential of *n*-alkanes for chemotaxonomic studies has been suggested based on the finding that the homologues C_{27} and C_{29} are sourced pre-

dominantly from trees and shrubs, whereas the homologues C_{31} and C_{33} are sourced predominantly from grasses and herbs (Maffei, 1996; Maffei et al., 2004; Rommerskirchen et al., 2006; Schäfer et al., 2016; Zech, 2009). Potential pitfalls when applying *n*-alkane proxies in paleovegetation studies should not be overlooked. For instance, (Bush and McInerney, 2013) caution against the chemotaxonomic application of *n*-alkanes because of high *n*-alkane pattern variability within graminoids and woody plants (Schäfer et al., 2016) emphasized the need for establishing regional calibration studies and Zech et al., (2011a, 2013) point to degradation affecting *n*-alkane proxies.

While the overall aim of our research is to contribute to the reconstruction of the paleoclimate and environmental history of the Bale Mountains, this study focuses more specifically on the following questions: (i) do lignin phenols and *n*-alkane biomarkers allow a chemotaxonomic differentiation of the dominant plant types of the Bale Mountains? (ii) Are the biomarker patterns of the plants reflected by and incorporated into the respective soils? (iii) Which implications have to be drawn from those results for planned paleovegetation reconstructions in the study area, e.g., concerning the reconstruction of the former extent of *Erica*? Finally, improved knowledge of the vegetation history of the Bale Mountains may help to support the biodiversity conservation program of the park in the face of future climate change and increasing human pressure.

2 Material and methods

2.1 Study area and sample description

The Bale Mountains are located 400 km southeast of Addis Ababa, the capital of Ethiopia (Hillman, 1986). Geographically, they belong to the Bale–Arsi massif, which forms the western section of the southeastern Ethiopian Highlands (Hillman, 1988; Mieke and Mieke, 1994; Tiercelin et al., 2008). The Bale Mountains National Park (BMNP) is situated at $39^{\circ}28'$ to $39^{\circ}57'$ E and $6^{\circ}29'$ to $7^{\circ}10'$ N, (Hillman, 1988; Mieke and Mieke, 1994; Umer et al., 2007) with elevations ranging from 1400 to 4377 m a.s.l. The highest part forms the Sanetti Plateau, on which the second highest peak of the country, Mount Tullu Dimtu at 4377 m a.s.l. is also located (Hillman, 1988). The plateau is limited by the steep Hareenna escarpment in the south and the southeast. The northeastern part is encompassed by high ridges and broad valleys that gradually descend towards the extensive Arsi–Bale plateaus and further into the Central Rift Valley lowlands (Hillman, 1988; Tiercelin et al., 2008). The topography of the Bale Mountains results in climatic gradients with respect to spatial and temporal distribution of rainfall as well as temperature (Tiercelin et al., 2008). Mean maximum temperature (MMT) on the mountain peaks ranges between 6 and 12 °C. At Dinsho (head-quarters, 3170 m a.s.l.) the MMT is 11.8 °C. Mean mini-

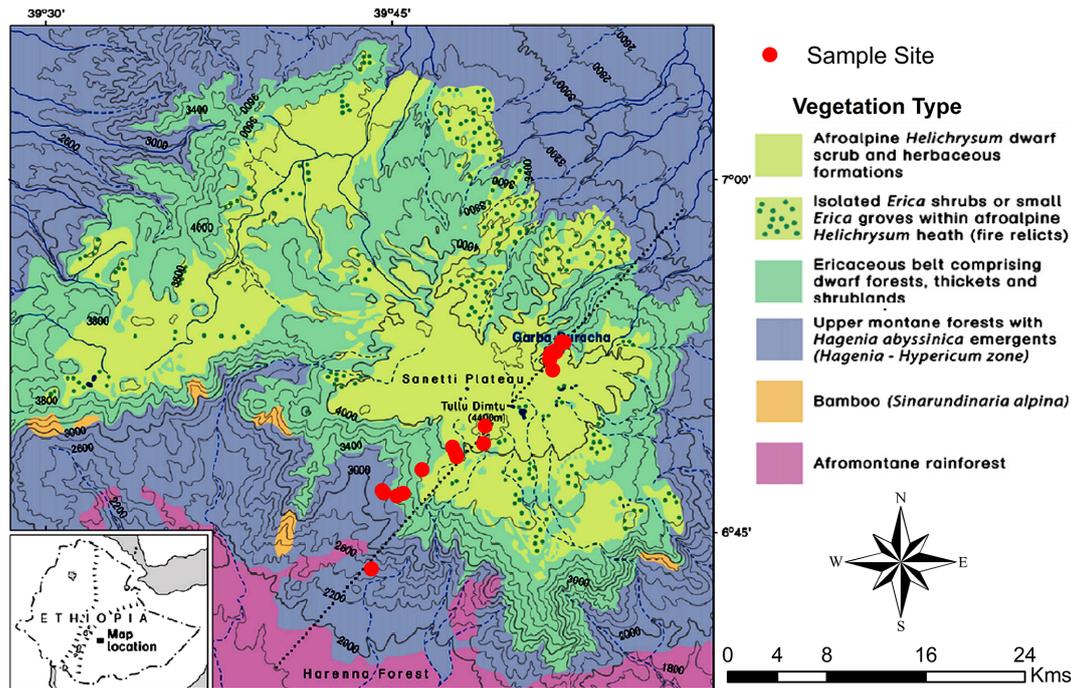


Figure 1. Map of the Bale Mountains for the vegetation zones and study sites along the northeastern and the southwestern transect (modified following Miehe and Miehe, 1994). Dominant vegetation types sampled that comprise the Ericaceous and afroalpine belt.

imum temperature ranges from 0.6 to 10 °C, with frequent frost occurring in the high peak areas during the winter season (Tiercelin et al., 2008). The highest annual rainfall and humidity occurs in the southwest part of the mountain with 1000–1500 mm yr⁻¹, and the northern part of the mountains exhibits annual rainfall ranging between 800 and 1000 mm yr⁻¹ (Woldu et al., 1989). The vegetation shows an altitudinal zonation comprised of the afromontane rainforest (1450–2000 m a.s.l.), the upper montane forests dominated by *Hagenia* and *Hypericum* species (2000–3200 m a.s.l.); the Ericaceous belt (3200–3800 m a.s.l.); and the afroalpine zone (3800–4377 m a.s.l.) dominated by dwarf shrubs such as *Helichrysum*, *Alchemilla*, herbs, and grasses (mostly *Festuca*; Fig. 1) (Friis, 1986; Miehe and Miehe, 1994). Geologically, the Bale Mountains consist of a highly elevated volcanic plateau dominated by alkali basalt, tuffs and rhyolite rocks. During the Last Glacial Maximum (LGM), it is understood that the regions of the high peak summits were glaciated and later flattened by repeated glaciations (Kidane et al., 2012; Osmaston et al., 2005; Umer et al., 2004). The soils having developed on the basaltic and trachyte rocks can be generally characterized as silt loam, having a reddish brown to black color (Woldu et al., 1989). They are usually shallow, gravelly and are assumed to have developed since the glacial retreat (Hedberg, 1964). Andosols are the most ubiquitous soil types. Nevertheless, Cambisols and Leptosols are also prevalent soil types in some parts of the Bale Mountains. In

wetland and sedimentary basins, Gleysols and Histosols are also common (Billi, 2015; Yimer et al., 2006).

In February 2015, 25 leaf and twig samples of the dominant plant species were collected (Fig. 1) along a southwestern and a northeastern transect (ranging from 2550 to 4377 m a.s.l. and 3870 to 4134 m a.s.l., respectively). Samples comprised of *Erica trimera* (Engl.) Beentje ($n = 5$), *Erica arborea* L. ($n = 5$), *Alchemilla haumannii* Rothm. ($n = 5$), *Festuca abyssinica* Hochst. ex A. Rich. ($n = 6$), *Helichrysum splendidum* Thunb. L. ($n = 2$), *Kniphofia foliosa* Hochst. ($n = 1$) and *Lobelia rhynchopetalum* Hemsl. ($n = 1$). Additionally, 15 organic surface layers (= O layers, strongly humified plant residues) and 22 mineral topsoils (= A_h horizons) that developed under the above listed dominant vegetation were collected from 27 sampling sites, resulting in 62 samples in total. For photos illustrating the investigated plant species and typical study sites, the reader is referred to Fig. 2 of our companion paper by Mekonnen et al. (2019). All samples were air-dried in the Soil Store Laboratory of the National Herbarium, Department of Plant Biology and Biodiversity Management, Addis Ababa University. In the laboratories of the Soil Biogeochemistry Group, Martin Luther University of Halle-Wittenberg, soil samples were sieved using a mesh size of 2 mm, finely ground, homogenized and subjected to further biogeochemical analysis.

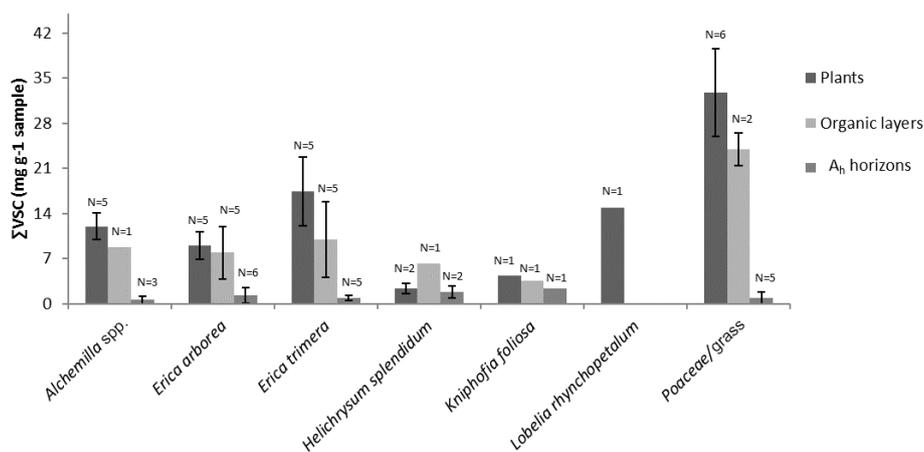


Figure 2. Sum of lignin phenol concentrations (Σ VSC) of contemporary plants, O layers and A_h horizons. Error bars illustrate standard deviations.

2.2 Analysis of lignin-derived phenol and leaf-wax-derived *n*-alkane biomarkers

Lignin phenols were extracted from 35, 50 and 500 mg of plant, O-layer and A_h-horizon soil samples, respectively. The analytical procedure followed the cupric oxidation (CuO) method developed by Hedges and Ertel (1982) and modified later on by Goñi and Hedges (1992). Briefly, the samples were transferred into Teflon digestion tubes together with 100 mg of (NH₄)₂Fe(SO₄)₂ × 6H₂O, 500 mg of CuO, 50 mg of C₆H₁₂O₆, 1 mL of ethylvanillin solution (100 ppm) as internal standard 1 (IS1) and 15 mL of 2M NaOH and digested at 170 °C for 2 h under pressure. Reaction products were cooled overnight and transferred into centrifuge tubes. Then the phenolic compounds were purified by adsorption on C₁₈ columns, desorbed by ethylacetate and concentrated under a stream of nitrogen gas for 30 min. Residue was dissolved in 1 mL phenylacetic acid (PAA), a working internal standard stock solution to determine the recovery of ethylvanillin before derivatization (Amelung et al., 2002; Möller et al., 2002). Finally, the samples were derivatized using 200 μL of N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 100 μL of pyridine. Oxidation products of lignin phenols were quantified using a SHIMADZU QP 2010 gas chromatography (GC) instrument coupled with a mass spectrometer (MS), (GCMS–QP2010, Kyoto, Japan).

After recovery correction, the concentration of each lignin phenol (in mg g⁻¹) was calculated from two or three CuO oxidation products according to the Eqs. (1), (2) and (3), respectively.

$$\text{Vanillyl (V)} = \text{vanillin} + \text{acetovanillone} + \text{vanillic acid} \quad (1)$$

$$\begin{aligned} \text{Syringyl (S)} &= \text{syringaldehyde} + \text{acetosyringone} \\ &+ \text{syringic acid} \end{aligned} \quad (2)$$

$$\text{Cinnamyl (C)} = p\text{-coumaric acid} + \text{ferulic acid} \quad (3)$$

For data evaluation, the sum of V, S, and C (Σ VSC); the ratios of S / V, and C / V; and the ratios of acids to aldehydes (Ac / Al) for the syringyl and vanillyl units were additionally calculated.

Leaf-wax-derived *n*-alkanes were extracted from 0.5 to 1 g of plant, O-layer and A_h-horizon soil samples using Soxhlet extraction by adding 150 mL of dichloromethane (DCM) and methanol (MeOH) as solvents (9 : 1 ratio) for 24 h following a method modified following Zech and Glaser (2008). In brief, 50 μL of 5 α -androstane were added to the total lipid extracts (TLEs) as internal standard. TLEs were concentrated using rotary evaporation and transferred to aminopropyl columns. Three lipid fractions containing the *n*-alkanes, alcohols and fatty acids, respectively, were eluted successively by using 3 mL of hexane, DCM / MeOH (1 : 1), and diethyl ether and acetic acid (95 : 5) as eluent. The *n*-alkanes were separated on a gas chromatograph (GC) and detected by a flame ionization detector (FID), whereas the other two lipid fractions (alcohols and fatty acids) were archived. The GC instrument (GC–2010 SHMADZU) was equipped with a SPB–5 column (28.8 m length, 0.25 mm inner diameter, 0.25 μm film thickness). The injector and detector temperature were 300 and 330 °C, respectively. The initial oven temperature was 90 °C. It is then raised in three ramps to 250 °C at 20 °C min⁻¹, further to 300 °C at 2 °C min⁻¹ and finally to 320 °C at 4 °C min⁻¹, resulting in a total oven runtime of 50 min. Helium (He) was used as carrier gas and *n*-alkane mixture (C₈–C₄₀) was used as external standard for peak identification and quantification.

The total *n*-alkane concentration (TAC), the average chain length (ACL, following Poynter et al., 1989) and the odd over even predominance (OEP, following Hoefs et al. (2002), the latter being very similar to the carbon preference index (CPI), were calculated according to the Eqs. (4), (5) and (6), respectively.

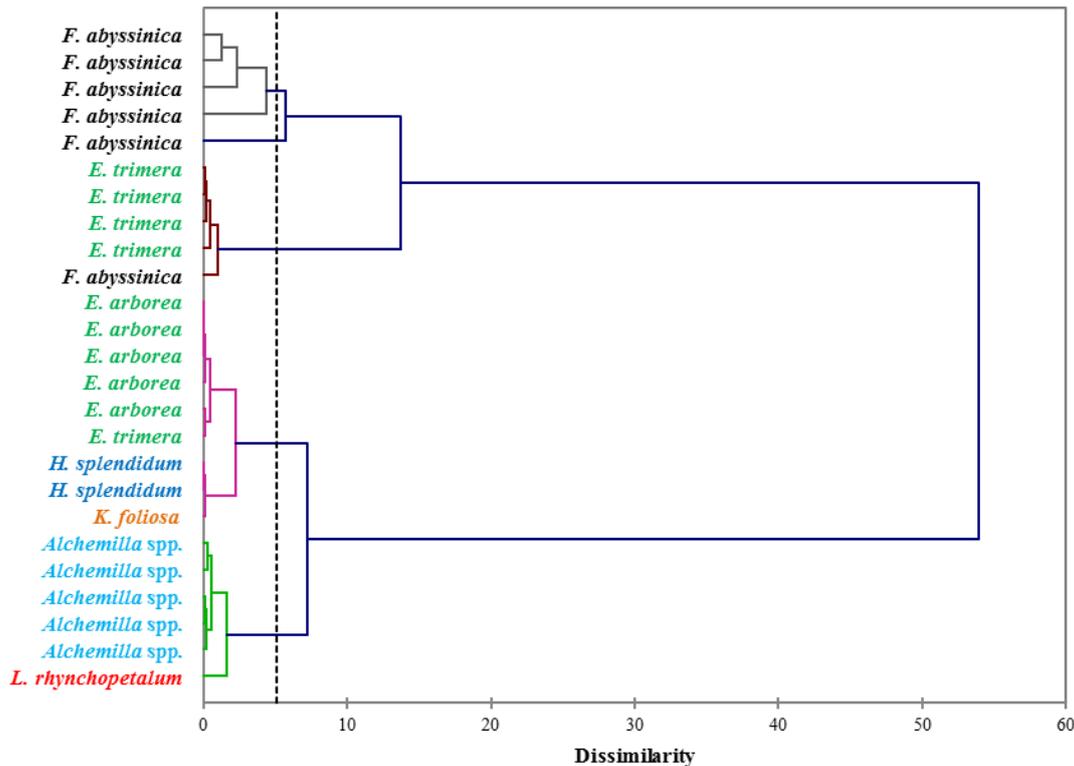


Figure 3. Dendrogram differentiating the dominant plant species of the Bale Mountains based on the concentrations of vanillyl, syringyl and cinnamyl lignin phenols (mg g^{-1} sample). The dotted vertical line represents the distance or dissimilarity between clusters.

$$\text{TAC} = \sum C_n, \text{ with } n \text{ ranging from 25 to 35,} \quad (4)$$

$$\text{ACL} = \sum(C_n \times n) / \sum C_n, \quad (5)$$

where n refers to the odd numbered n -alkanes ranging from 27 to 33

$$\text{OEP} = (C_{27} + C_{29} + C_{31} + C_{33}) / (C_{26} + C_{28} + C_{30} + C_{32}). \quad (6)$$

All calibrated datasets of the analytical results were subjected to simple correlation test and agglomerative hierarchical clustering (AHC) using XLSTAT (2014) statistical software. R software version 3.4.2 was also used to demonstrate taxonomic differences and the effect of biodegradation on the sample materials via ternary diagrams and notched box plots.

3 Results and discussion

3.1 Lignin phenol concentration and patterns of contemporary vegetation

The $\sum\text{VSC}$ of modern plants investigated from the two transects of the Bale Mountains ranges from 1.8 to 41.8 mg g^{-1} , the sample with *Festuca* yielding the highest average contribution to TOC with up to 33 mg g^{-1} sample (Fig. 2). This is within the range reported in the literature (Belanger et al.,

2015; Hedges et al., 1986). Note that lignin phenol concentrations of grasses are higher compared to other vegetation of the Bale Mountains, although it is known that grasses contain only low amounts of lignin when compared to trees.

The concentrations of individual lignin phenols (vanillyl, syringyl and cinnamyl) allow us to chemotaxonomically differentiate the contemporary dominant plant species of the Bale Mountains. This is illustrated in Fig. 3, based on an agglomerative hierarchical cluster analysis (AHC). The abundance of individual lignin phenols (V, S and C) was specific to individual or restricted groups of plant and/or tissues applied to cluster different taxa (Belanger et al., 2015; Castañeda et al., 2009; Goñi and Hedges, 1992; Hedges and Mann, 1979; Tareq et al., 2004, 2006).

While Fig. 3 highlights the potential for chemotaxonomic differentiation of the investigated plants, it does not yet become clear from this hierarchical cluster analyses result which lignin phenols are characteristic for which plants and which lignin proxy might have potential for paleovegetation reconstructions. Therefore, Fig. 4 shows the relative abundance of V, S and C for all investigated plant species in a ternary diagram. Accordingly, *Erica arborea* and *Erica trimera* are characterized by cinnamyl percentages of > 40 %, whereas, except for two *Festuca* samples, all other plants are characterized by cinnamyl percentages of < 40 %. Our results from fresh plant material are hence not in agree-

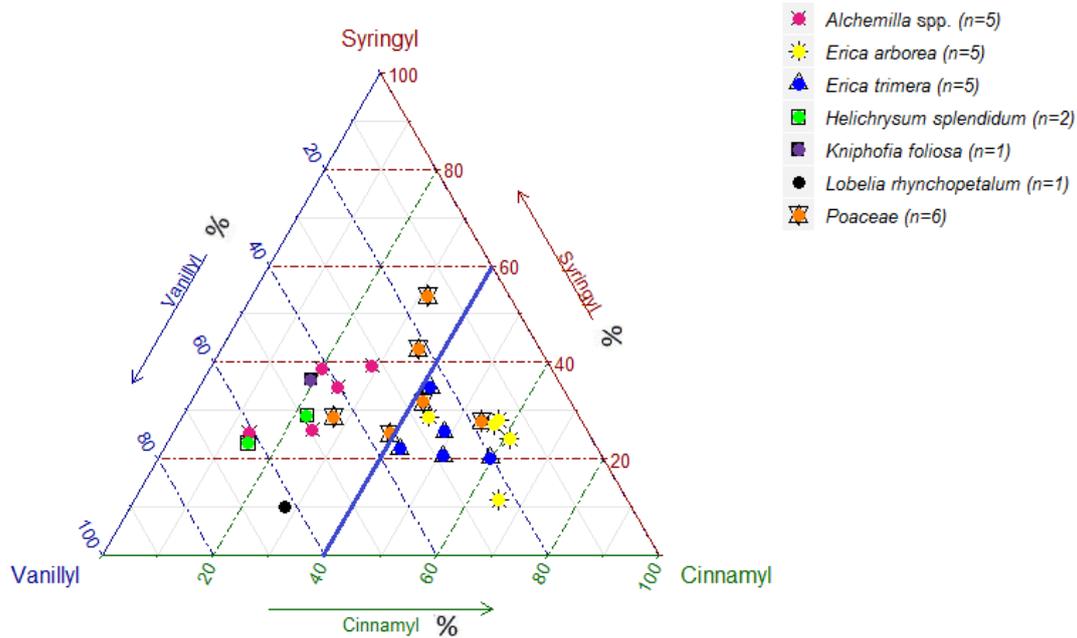


Figure 4. Ternary diagram for the relative abundances (%) of vanillyl, syringyl and cinnamyl lignin phenols of the dominant vegetation. The blue line separates samples with more (right) versus less (left) than 40 % cinnamyl.

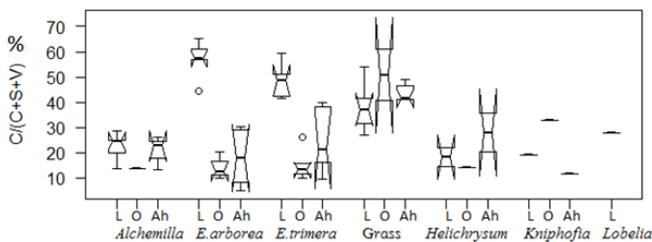


Figure 5. Box plot for the relative abundance of cinnamyl phenols (expressed as $C / (V + S + C)$ in %) in plants, O layers and A_h horizons. The box plots indicate the median (solid line between the boxes) and interquartile range (IQR), with upper (75 %) and lower (25 %) quartiles and possible outliers (white circles). The notches display the confidence interval around the median within $\pm 1.57 \times \text{IQR}/\text{sqrt}$. Note that small sample sizes result in unidentifiable boxes (particularly *Kniphofia* and *Lobelia*).

ment with the finding of Hedges and Mann (1979) and Hedges et al. (1988) that high contributions of cinnamyl phenols are characteristic of non-woody grass and fern. Despite a relatively large scattering and a partial overlapping, our results suggest that the ratio $C / (V + S + C)$ might be used as a proxy for distinguishing *Erica* spp. from other vegetation types of the Bale Mountains, with values > 0.40 being generally characteristic for *Erica* spp.

3.2 Lignin phenol patterns of O layers and A_h horizons

$\sum\text{VSC}$ strongly decreases from plants over O layers to A_h horizons, except for *Helichrysum*, which yielded the lowest $\sum\text{VSC}$ values of all plants (Fig. 2). This descending trend is in agreement with the literature (Amelung et al., 1997; Belanger et al., 2015) and reflects the preferential degradation of the plant-derived lignin phenols compared to other soil organic matter constituents. At the same time, the input of root-derived lignin is very likely. As a result of both processes, i.e., degradation and lignin input by roots and the large and chemotaxonomically characteristic contribution of C in *Erica* plant material ($\geq 41.5\%$) is lost in the O layers ($C < 27\%$), whereas the two investigated O layers under *Festuca* yielded relative C contributions $> 40\%$. Similarly, A_h horizons under *Festuca* are characterized by C contributions $> 40\%$, while all A_h horizons that developed under other vegetation types are characterized by C contributions $< 40\%$. This finding does not ad hoc preclude the above proposed lignin phenol proxy $C / (V + S + C)$ for reconstructing vegetation history, but it definitely challenges its application. Degradation and lignin input by roots need to be considered when interpreting phenol proxies. This is relevant beyond our case study concerning *Erica* versus *Festuca* and *Helichrysum* (Fig. 5) and is likely more relevant in paleosols than in sedimentary archives.

In our study, we found no consistent increase and systematic relationship between A_c / A_l ratios of V and S, which are used as degradation proxies in some studies (Amelung et al., 2002; Hedges and Ertel, 1982; Möller et al., 2002; Tareq

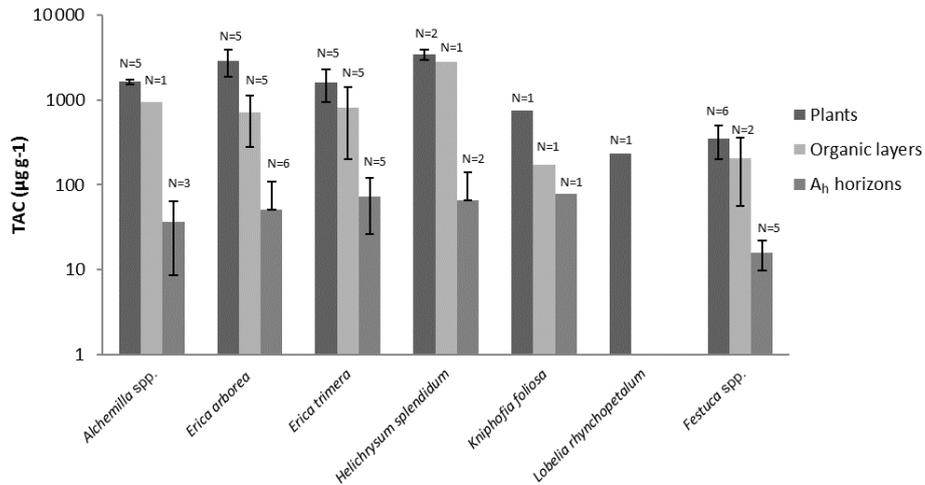


Figure 6. Total long-chain *n*-alkane concentrations (TACs) of plants, O layers and A_h horizons. Error bars illustrate standard deviations.

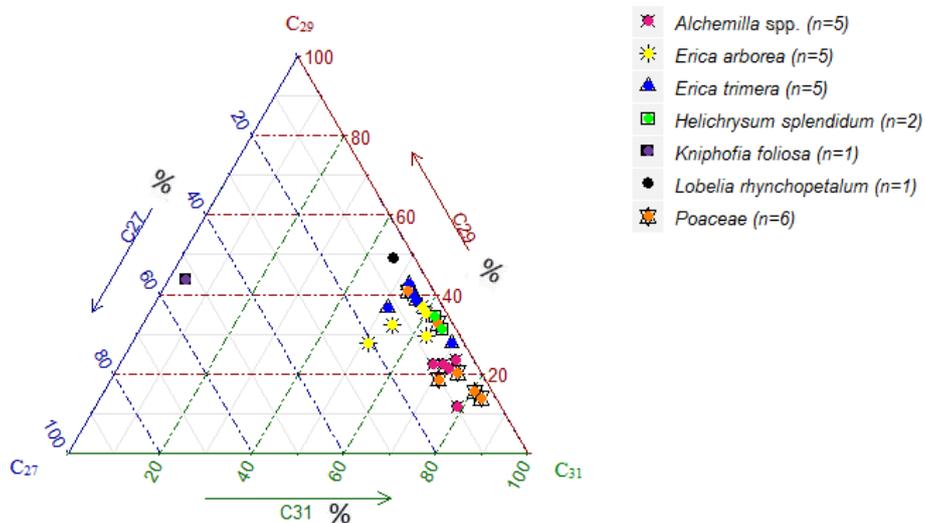


Figure 7. Ternary diagram illustrating the relative abundance (%) of the *n*-alkanes C_{27} , C_{29} and C_{31} in the investigated plant samples.

et al., 2011), and source proxy (S/V). This is in agreement with other studies (Belanger et al., 2015), and we therefore suggest that caution needs to be taken when using Ac/Al ratios as degradation proxies.

3.3 *n*-alkane concentrations and patterns of contemporary plants

To characterize the dominant plant species chemotaxonomically, *n*-alkanes with a chain length of 21–37 C atoms were considered as characteristic for epicuticular leaf waxes, typical for higher plants (Eglinton and Hamilton, 1967; Hoffmann et al., 2013). Most of the investigated plant species showed total *n*-alkane concentrations (TAC, C_{25} – C_{35}) above $800 \mu\text{g g}^{-1}$. Only *Lobelia* and *Festuca* exhibited total *n*-alkane concentrations below $800 \mu\text{g g}^{-1}$ (Fig. 6). The TAC values of the O layers were only slightly lower when com-

pared to contemporary plants. By contrast, the TAC values of the A_h horizons were significantly lower compared to contemporary plants (Fig. 6). The *n*-alkane concentrations in this study are in agreement with research findings for fresh plant materials (Bush and McInerney, 2013; Feakins et al., 2016) and soils (Schäfer et al., 2016).

Contrary to lignin phenols, hierarchical cluster analysis of individual *n*-alkanes did not allow for unambiguous differentiation between *Erica* and non-*Erica* species. Therefore, the *n*-alkane patterns do not allow for developing a proxy for identifying *Erica*, at least in the Bale Mountains. Average chain length values (ACLs) of plant and soil *n*-alkanes range between 28 to 32 and 29 to 31, respectively. The ACLs of *Erica arborea* (30.5) and *Erica trimera* (30.5) are identical, which could be explained by the monophyletic origin of the species (Guo et al., 2014). Grass sam-

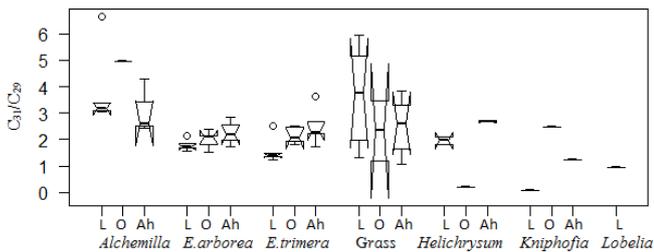


Figure 8. Box plot for the ratio C_{31}/C_{29} in plant samples, organic layers and A_h horizons. The box plots indicate the median (solid line between the boxes) and interquartile range (IQR), with upper (75%) and lower (25%) quartiles and possible outliers (white circles). The notches display the confidence interval around the median within $\pm 1.57 \times \text{IQR}/\text{sqrt}$. Note that small sample sizes result in unidentifiable boxes (particularly *Kniphofia* and *Lobelia*).

ples (*Festuca abyssinica*) exhibited a clear predominance of C_{31} (Fig. 7), which was reported before by different authors (Schäfer et al., 2016; Zech, 2009). Most other investigated plant species revealed a predominance of either C_{29} or C_{31} . Only *Kniphofia foliosa* is characterized by high relative abundance of C_{27} and C_{29} , while C_{31} is almost absent (Fig. 7).

Apart from individual *n*-alkanes, the ratio of C_{31}/C_{29} depicts *Erica* litter as significantly distinguishable from the other species, except for *Helichrysum splendidum* (Fig. 8).

3.4 *n*-alkane concentration and pattern of O layers and A_h horizons

The TAC values decrease in the following order: plants > O layers > A_h horizon (Fig. 6). The odd over even predominance values (OEPs) of the plants, O layers and A_h horizons range from 5 to 90 ($\bar{x} = 21$), 4 to 42 ($\bar{x} = 15$) and 2 to 37 ($\bar{x} = 16$), respectively. The OEP values of the plants (which are almost identical to the CPI values) are therefore well within the range reported (Diefendorf et al., 2011) for angiosperms. Decreasing OEP values towards O layers and A_h horizons are often observed and can be explained with organic matter degradation (Schäfer et al., 2016; Zech et al., 2011b). The still relatively high OEP values ($\bar{x} = 16$) obtained for the topsoils of our study area indicate that the *n*-alkanes are not strongly degraded. Importantly, *n*-alkane degradation affects not only the OEP values but also the *n*-alkane ratios, such as the above presented ratio C_{31}/C_{29} that allows distinguishing *Erica* from non-*Erica* vegetation. As a result, this ratio in particular no longer allows for chemotaxonomically distinguishing between soils that have developed under *Erica* versus *Alchemilla* and grass (Fig. 8). Unlike with lignin phenols, a noteworthy influence from root-derived *n*-alkanes on O layers and A_h horizons can be excluded. This is based on the notion that roots contain lower *n*-alkane concentrations by several magnitudes than above-ground plant material and results from studies using

^{14}C dating of *n*-alkanes in loess–paleosol sequences (Häggi et al., 2014; Zech et al., 2017).

4 Conclusions and implications for paleovegetation reconstructions in the Bale Mountains

One of the premises within the Mountain Exile Hypothesis project (DFG-FOR 2358) is to reconstruct the dynamics of *Erica* vegetation on the Sanetti Plateau in the Bale Mountains National Park, Ethiopia. While our companion paper by Mekonnen et al. (2019) focused on stable carbon and nitrogen isotopes and hemicellulose-derived sugar biomarkers, we tested in this regional calibration study the potential of cupric oxide lignin phenols and leaf-wax-derived *n*-alkanes to serve as unambiguous proxies for differentiating between *Erica* versus non-*Erica* vegetation.

A hierarchical cluster analysis of individual lignin phenols was promising and allowed the chemotaxonomic differentiation of *Erica* from non-*Erica* vegetation based on relatively high relative contribution of cinnamyl ($\geq 40\%$) phenols. However, this characteristic pattern is not reflected in the O layers and A_h horizons. In all likelihood, the loss of the cinnamyl dominance is caused by preferential degradation. Unlike expected, we found no overall evidence for increasing $(\text{Ac}/\text{Al})_{\text{v+s}}$ ratios as a proxy for degradation from plant material over O layers to A_h horizons.

Erica could not be differentiated chemotaxonomically from all other investigated plant species using *n*-alkanes in a hierarchical cluster analysis. Nevertheless, *Erica* was still characterized in our dataset by significantly lower C_{31}/C_{29} ratios compared to *Alchemilla* and grasses. However, like lignin-derived phenol proxies, the *n*-alkane patterns are changing due to degradation from plant material over O layers to A_h horizons, thus inhibiting their application for an unambiguous chemotaxonomic identification of *Erica* in soils and sediments. Therefore, future work is planned focusing on alternative molecular markers such as tannin-derived phenols and terpenoids.

Data availability. The underlying datasets used in this study are accessible via <https://doi.org/10.5281/zenodo.3372104>.

Author contributions. BG, WZ and MZ developed the project idea in collaboration with SN and TB. WZ, BL and BM designed and handled field research work. BL and LB performed the laboratory work. The manuscript was prepared by BL with the support of MZ and the other co-authors.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgements. We are grateful to the Ethiopian Biodiversity Institute, Ethiopian Wildlife Conservation Authority, Bale Mountains National Park and Addis Ababa University for providing us with access to the plant genetic resources, access to the Bale Mountains National Park and for their scientific collaboration, respectively. We acknowledge the financial support within the funding program Open Access Publishing by the German Research Foundation (DFG). Bruk Lemma expresses his gratitude to the Catholic Academic Exchange Services (KAAD, Germany) for the PhD scholarship and his sincere thanks to Heike Maennicke for kind assistance during the laboratory work. We kindly thank three anonymous reviewers for their constructive comments and suggestions that helped us to improve the quality of this paper.

Financial support. This research has been supported by the DFG within Research Unit “The Mountain Exile Hypothesis” (grant nos. GL 327/18-1, ZE 844/10-1 and ZE 154/70-1).

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