

Dear Editor,

5 we would like to thank you for your effort with our manuscript and the constructive comments/suggestions of both reviewers. We have revised the manuscript according to the reviewer suggestions. Please find below our point-by-point reply and the manuscript with marked changes.

With best wishes on behalf of the co-authors,

10 Marcel Bliedtner

Anonymous Reviewer #1

15 1. >>A more detailed dataset of environmental and climatic parameters, such as temperature, humidity (aridity), precipitation, etc., along the sampling transect, or even for each sampling site, is needed. These data should be examined to see whether and how they influence the n-alkane compositions in general. Authors are encouraged to discuss separate roles of vegetation and environment in modulating n-alkane distributions. For example, as noted in the text, samples 3p, 9p, 25p, 29p, 34p do not show composition patterns as expected. The authors think that these samples may have been influenced by climate. However, the interpretation is rather qualitative and unclear. I guess some  
20 plant samples of the same species may have been distributed along climatic gradients. If so, data of these samples are valuable and should be sorted out to see their possible responses to climatic change.<<

We agree that climate can play an important role in the leaf wax distribution. To address those issues, we will correlate environmental data, namely mean annual temperatures and precipitation, with our n-alkane distribution from the investigated sites. However, climate data for eastern Georgia are scarce because only few climate stations are located  
25 within the study area, and almost all stations show large data gaps after 1990. So, reliable climate data cannot be derived from climate stations along the investigated transect or even for each sampled site. We therefore used mean annual temperature data of the climate station Tbilisi that is located at 490 m a.s.l.. Based on this reference station, mean annual temperatures for our investigated sites were calculated under the assumption that temperature declines linearly with 0.65 K by 100 m increase in elevation. For precipitation, we used an unpublished precipitation map of Georgia (V. Bagrationi Geographical Institute Tbilisi). We also tried the WorldClim – Global Climate Data (Fick and  
30 Hijmans, 2017) that provides interpolated average monthly climate data with a spatial resolution of 1 km<sup>2</sup>. However, robust temperature and precipitation data could not be derived for eastern Georgia from that dataset, most likely due to the coarse spatial resolution.

35 2. >>The degradation lines in figure 5 are interesting. But it is obvious that the data are much scattering. I would like to see more discussion on the causes of the scattering, including, e.g., climatic factors, disproportional input of leaf waxes to soils from different plants. Also, if the causes are significant, the authors should admit the weakness of the end-member model.<<

Based on our correlation with environmental factors, we will include a more comprehensive discussion about possible climate-induced causes of end-member scattering, as well as species-specific causes and degradation effects.

40 3. >>As the authors stated in the text, this study is region specific and results appear different from other regions and the globe. It is expected that a comparison of this work with others, and hence a more comprehensive study may improve this paper and is greatly helpful for readers. I suggest the authors give a try.<<

A more detailed comparison of our results with other regions will be included.

Some technical corrections:

45 1. L16-17, P1. “temperate and tropical regions” can also be “semi-humid to semi-arid”. Rephrase it.

- Will be rephrased.
- 2. L13, P4. “C25 and C35”? I guess “and” should be “to”.
  - Will be done so.
- 3. L16 &18, P5. ACL and OEP have been explained in previous text, so no need of full names.
  - Will be done so.
- 5
- 4. L5, P6. “What” seems incorrect, maybe “which”.
  - Will be done so.

## References

- 10 Fick, Stephen E.; Hijmans, Robert J. (2017): WorldClim 2. New 1-km spatial resolution climate surfaces for global land areas. In: *Int. J. Climatol* 37 (12), S. 4302–4315. DOI: 10.1002/joc.5086.

## Anonymous Reviewer #2

- 15 1. >>In Fig.3, larger differences seem appeared in the ACL (why not used) whilst equal values occurred in OEP. Obviously, the ACL values were not used. Its relationship between ACL and n-alkane ratios should be provided in Fig.5, as least provide in the supplementary materials.<<
- 20 Like the n-alkane ratio, the ACL show distinct differences in the n-alkane distribution from grass and deciduous sites and is thus suitable to describe differences in the vegetation composition. We will provide a comparison of both ratios used for the end-member model (i.e. Fig. 5) in the supplementary materials. However, compared to the ACL, the n-alkane ratio shows greater discrimination power in the end-member model between grasses and deciduous trees and seems more suited for describing vegetation differences along our investigated transect.
2. >>In Fig. 4, it seems that similar distribution occurred on the modern plant and topsoil. For a better effect, grassland and deciduous site are suggested to be classified rather than between plant and topsoils.<<
- Will be done so.
- 25 3. >>In Fig.5, the degradation line for grassland seems perfect but is not ok for the deciduous-site if the logic from plant to soil is implemented, which is usually decreases with low OEPs and with a converging degradation line. But it is not the case, such as 35p!35s, 20p!20s, 16p!16s, as well as 9p!9s, 23p!23s, 34p!34s. It seems complicated in the deciduous site for the degradation of OM.<<
- 30 We agree that OEP values should decrease from plants to subsoils, i.e. with ongoing degradation. Up to now, we do not fully understand the degradation of leaf wax n-alkanes in soils, but we will include a more comprehensive discussion about degradation effects.
4. >>According to the results, the potential regional paleoenvironmental reconstructions should be limited to the paleovegetation as illustrated in title, this should be careful in the abstract and conclusion part.<<
- 35 Will be done so.

# Leaf wax *n*-alkanes in modern plants and topsoils from eastern Georgia (Caucasus) – implications for reconstructing regional paleovegetation

Marcel Bliedtner<sup>1,2</sup>, Imke K. Schäfer<sup>2</sup>, Roland Zech<sup>1,3,2</sup>, Hans von Suchodoletz<sup>3</sup>

5 <sup>1</sup>~~Institute~~<sup>1</sup>Institute of Geography, University of Jena, Löbdergraben 32, D-07743 Jena, Germany

<sup>2</sup>~~Institute~~<sup>2</sup>Institute of Geography and Oeschger Centre for Climate Change Research, University of Bern, Hallerstrasse 12, CH-3012 Bern, Switzerland

<sup>3</sup>~~Institute~~<sup>3</sup>Institute of Geography, University of Leipzig, Johannisallee 19a, D-04103 Leipzig, Germany

<sup>3</sup>~~Institute of Geography, University of Jena, Löbdergraben 32, D-07743 Jena, Germany~~

10 Correspondence to: Marcel Bliedtner (marcel.bliedtner@~~giub.unibe.ch~~uni-jena.de)

## Abstract.

~~Long-chain *n*-alkanes became increasingly used for paleoenvironmental studies during the last years as they have the great potential to reconstruct past changes in vegetation and climate. They mostly~~Long-chain *n*-alkanes originate from leaf waxes of higher terrestrial plants, are relatively resistant against physical and chemical degradation and ~~can thus serve as valuable~~  
15 ~~biomarkers that~~ are preserved in ~~various sedimentary~~sediment archives for at least millennial timescales. ~~Since their~~ homologous patterns discriminate between vegetation forms, they were increasingly used for paleovegetation reconstructions ~~during the last years.~~ However, before any robust interpretation of the long-chain *n*-alkane patterns in ~~sedimentary~~sediment archives, reference samples from modern vegetation and topsoil material should be investigated at a regional scale. Apart from ~~Central and South Eastern Europe~~some temperate and tropical regions, such systematic regional studies on modern plant and  
20 topsoil material are still largely lacking.

To test the potential of leaf wax–derived *n*-alkane patterns for paleoenvironmental studies in the semi-humid to semi-arid ~~central~~ southern Caucasus region, we investigated the influence of different vegetation ~~types~~forms on the leaf wax *n*-alkane signal in modern plants and topsoil material (0-5 cm) from eastern Georgia. We sampled (i) sites with grassland/~~herbs~~ that included steppe, cultivated grassland and meadows, and (ii) sites that are dominated by deciduous hornbeam forests.

25 The ~~*n*-alkane~~ results show ~~distinct and systematic differences between samples~~that long-chain *n*-alkanes originate from ~~sites with the leaf waxes of higher terrestrial plants and that their homologous pattern allow to discriminate between~~ different vegetation ~~types~~forms: ~~*n*-alkanes~~Alkanes derived from sites with grassland/~~herbs~~ are mainly dominated by C<sub>31</sub>, while *n*-alkanes derived from sites with deciduous trees/~~shrubs~~ show high abundances of C<sub>29</sub>. Thus, ~~chain length ratios allow to discriminate between these two different vegetation types and long-chain *n*-alkanes~~ have a great potential when used for regional ~~paleoenvironmental~~paleovegetation reconstructions. ~~Moreover, the *n*-alkane distributions of the topsoils do not show correlations with mean annual temperatures and precipitation along the investigated transect.~~ As degradation of organic matter can affect the leaf wax *n*-alkane distribution, we further present an updated end-member model that includes our results, accounts for degradation effects and enables semi-quantitative reconstructions of past vegetation changes in the ~~central~~ southern Caucasus region.

## 1 Introduction

Long-chain *n*-alkanes (C<sub>25</sub>-C<sub>35</sub>) are produced as part of the epicuticular leaf waxes by terrestrial plants and can thus serve as valuable biomarkers (Eglinton et al., 1962; Eglinton and Hamilton, 1967; Kolattukudy and Walton, 1973). Typically, leaf wax *n*-alkanes show a distinct odd-over-even predominance (OEP) (Eglinton and Hamilton, 1967), and their relative odd homologue distribution might be used to differentiate between different vegetation forms. The chain-lengths C<sub>27</sub> and C<sub>29</sub> are mainly produced by deciduous trees and shrubs, while C<sub>31</sub> and C<sub>33</sub> mainly derive from grasses and herbs (Marseille et al., 1999; Zech et al., 2009; Schwark et al., 2002). Because of their low water-solubility, chemical inertness and relative persistence against physical and chemical degradation, they stay well preserved in soils and sedimentary archives, at least over millennial time-scales (Eglinton and Eglinton, 2008). Up to now, long-chain *n*-alkanes have increasingly been applied to various paleoenvironmental archives such as lacustrine and marine sediments and loess-paleosol sequences to reconstruct paleovegetation (Schwark et al., 2002; Schefuß et al., 2003; Zhang et al., 2006; Liu and Huang, 2005; Schatz et al., 2011; Zech et al., 2009; Schäfer et al., 2016a) in order to reconstruct paleovegetation and climate.

However, robust interpretations of long-chain *n*-alkanes in sedimentary archives need to can only be evaluated obtained by modern, regional calibration studies on modern plant and topsoil material, but such calibration studies are still largely lacking.

The need for regional calibration studies is underlined by the fact that study of Bush and McInerney (2013) that report no discrimination of modern vegetation patterns/forms by *n*-alkanes from different sites around the world using *n*-alkanes, and therefore they questioned whether *n*-alkane patterns are suitable to distinguish between different vegetation types at least/forms on a global scale. In contrast, on a although still lacking for many regions, some regional level some studies from Central and South-Eastern Europe prove and tropical South America and Africa demonstrate the discrimination power of *n*-alkanes for vegetation reconstruction by *n*-alkanes/reconstructions, although the most abundant *n*-alkane homologues show regional differences (Zech et al., 2009; Zech et al., 2013; Schäfer et al., 2016b)(Zech et al., 2009; Zech et al., 2013; Schäfer et al., 2016b; Feakins et al., 2016; Vogts et al., 2009): While Schäfer et al. (2016a/2016b) report that C<sub>27</sub> is as the most abundant homologue produced under by deciduous sites/trees/shrub vegetation in humid Central/Central Europe, Zech et al. (2013) show that C<sub>29</sub> is the dominant homologue under deciduous sites/produced by this vegetation form in more arid South-Eastern Europe. In tropical ecosystems, C<sub>29</sub> is the most abundant homologue in C3 rainforest trees, whereas C<sub>33</sub> is mainly produced by C4 grasses and herbs (Vogts et al., 2009; Feakins et al., 2016). Moreover, several potential pitfalls can complicate the interpretation of long-chain *n*-alkanes, as there are relatively wide species-specific variations (Diefendorf et al., 2011; Bush and McInerney, 2013) of the produced *n*-alkanes (Diefendorf et al., 2011; Bush and McInerney, 2013), environmental factors (Hoffmann et al., 2013; Tipple and Pagani, 2013)(Hoffmann et al., 2013; Tipple and Pagani, 2013) and degradation effects (Buggle et al., 2010) that all can additionally/potentially influence the *n*-alkane pattern. Thus/Therefore, prior to the application of long-chain *n*-alkanes for paleovegetation reconstructions in sedimentary archives, regional calibration sets from modern long-chain *n*-alkane patterns are necessary to i.) prove their leaf wax origin, and ii.) justify/assess their discrimination power between grasses/herbs and deciduous trees/shrubs.

This study evaluates long-chain *n*-alkane patterns of modern plant and topsoil material from eastern Georgia in the central-southern Caucasus region to test their ~~leaf wax origin and~~ chemotaxonomic potential, i.e. whether *n*-alkane abundances and chain-length distributions can be used to discriminate between deciduous trees/~~shrubs~~ and grasses/~~herbs~~ on a regional level. ~~Since climatic factors can potentially influence the *n*-alkane pattern, we checked such an influence by correlating mean annual~~  
5 ~~temperature and precipitation with the *n*-alkane distribution. Furthermore, ~~the~~ leaf wax *n*-alkane pattern can be affected by degradation effects, but these ~~effects~~ can be checked and corrected for with regionally derived end-members (Zech et al., 2013; Schäfer et al., 2016b). Thus, we further aim to establish a regional ~~calibration set and~~ end-member model of *n*-alkanes for the ~~central~~ southern Caucasus region as a base for future robust interpretations of leaf wax *n*-alkane patterns derived from regional sediment archives. Such lacustrine, ~~palustrine, fluvial, loess-paleosol or,~~ open-air archaeological ~~or fluvial-sediment~~ archives  
10 were increasingly investigated during the last years and could potentially be used ~~for *n*-alkane derived for~~ vegetation reconstructions ~~based on *n*-alkanes~~ (Messager et al., 2013; Joannin et al., 2014; Suchodoletz et al., 2015; Wolf et al., 2016; Egeland et al., 2016).~~

## 2 Material and Methods

### 2.1 Geographical setting and field sampling

15 The semi-humid to semi-arid central southern Caucasus ~~region~~ is characterized by a small-scale pattern of different ecologic regions. Due to a rain-shadow that is caused by the high elevation of the Western and Central ranges of the Greater Caucasus and by the ~~N-SNW-SE~~-directed Likhi Range linking the Greater and Lesser Caucasus, ~~(Fig. 1)~~, there exists a significant climatic gradient with decreasing precipitation and more continental conditions going eastwards (Connor and Kvavadze, 2008). Our study of modern plant and topsoil material ~~took place~~ ~~was carried out~~ in eastern Georgia (see Fig. 1) at the end of the  
20 vegetation period in September 2016. We sampled 22 sites that are located along a N-S-directed transect with a length of ca. 65 km. The transect extends from the surroundings of Tbilisi in the south over the mid-mountain Kura-fold-and-thrust-belt (Forte et al., 2010) into the upper part of the Alazani valley in the north. The sampled ~~sites with~~ grassland ~~sites/herbs~~ included steppe, cultivated grassland and meadows, and the ~~sites with~~ deciduous ~~sites/trees/shrubs~~ were mainly dominated by hornbeam forests (see Fig. 2A for sample locations and supporting online material SOM-1 and 2 for site descriptions). At each site, we  
25 sampled plant material and the upper 0-5 cm of the topsoil.

The altitudes along the transect range between 445 and 1659 m a.s.l., mean annual temperatures between ca. ~~11.35.4~~ and 13.3°C ([www.yr.no/place/Georgia/Tbilisi](http://www.yr.no/place/Georgia/Tbilisi); see chapter 2.3) and ~~recent~~ ~~mean~~ annual precipitation between ca. 600 and ~~1000~~ ~~2000~~ mm/a (unpublished precipitation map of W. Bagrationi Geographical Institute Tbilisi; Fig. 2C). Precipitation mainly falls in spring and early summer during convective events (Lydolph, 1977). The recent vegetation of eastern Georgia is part of the  
30 Irano-Turanian Group (Connor et al., 2004; Sagheb-Talebi et al., 2014). In the surroundings of Tbilisi, the natural vegetation is characterized by xerophytes and semidesert vegetation, while the vegetation on the southern slopes of the mid-mountain Kura-fold-and-thrust-belt changes from oak-hornbeam ~~forest~~ ~~forests~~ in the lower parts to mixed beech forests in the upper parts.

In the semi-humid lowlands of the upper Alazani valley, the natural vegetation can be classified as elm-oak-vine forests. However, agricultural fields cover most of the valley floor today. The mid-mountain belt of the upper Alazani valley is characterized by mixed beech and hornbeam forests and in small parts also by fir-spruce forests (Connor and Kvavadze, 2008, Fig. 2B).

## 5 2.2 Leaf wax analyses

### Analytical procedure

Leaf waxes were analysed at the Institute of Geography of the University of Bern/Switzerland. Free lipids from modern plant and topsoil samples were extracted using an ultrasonic treatment. Plant material (~1g) and soil material (~10g) were extracted three times with 20 ml dichloromethane (DCM): methanol (MeOH) (9:1, v/v) in an ultrasonic bath for 15 min.

10 The total lipid extract was separated over aminopropyl pipette columns into: i) the apolar fraction including the *n*-alkanes, ii) the polar fraction, and iii) the acid fraction. The *n*-alkanes were eluted with ~4 ml hexane and further purified over coupled silver-nitrate (AgNO<sub>3</sub>-) - zeolite pipette columns. The *n*-alkanes trapped in the zeolite were subsequently dissolved in hydrofluoric acid and recovered by liquid-liquid extraction using *n*-hexane.

Afterwards, the *n*-alkanes were identified and quantified using a gas chromatograph with a flame ionisation detector (GC-15 FID). GC-FID measurements were performed on an Agilent 7890 gas-chromatograph equipped with an Agilent HP5MS column (30 m \* 320 μm \* 0.25 μm film thickness). For quantification and identification, external *n*-alkane standards (C<sub>21</sub> – C<sub>40</sub>) were run with each sequence.

### Data analyses

*n*-Alkane concentrations were calculated as the sum of C<sub>25</sub> ~~and to~~ C<sub>35</sub>, and given in μg g<sup>-1</sup> dry weight.

20 Odd-over-even predominance (OEP) values (Eq. 1) were determined following Hoefs et al. (2002). Low values (<5) indicate an enhanced state of degradation (Bugge et al., 2010; Zech et al., 2009).

$$\text{OEP} = \frac{C_{27} + C_{29} + C_{31} + C_{33}}{C_{26} + C_{28} + C_{30} + C_{32}} \quad (1)$$

The average chain length (ACL) of *n*-alkanes (Eq. 2) was calculated after Poynter et al. (1989), and was used to distinguish between leaf waxes that predominantly originate from deciduous trees and shrubs (C<sub>27</sub> and C<sub>29</sub>; i.e. lower values) and those  
25 mainly originating from grass/herb vegetation (C<sub>31</sub> and C<sub>33</sub>; i.e. higher values).

$$\text{ACL} = \frac{27 * C_{27} + 29 * C_{29} + 31 * C_{31} + 33 * C_{33}}{C_{27} + C_{29} + C_{31} + C_{33}} \quad (2)$$

The *n*-alkane ratio (~~Eq. 3~~that is used for the end-member model to correct for degradation effects (Zech et al., 2013)) is a normalized ratio to differentiate between vegetation forms, with higher values for grasses and herbs and lower values for deciduous trees and shrubs: (Eq. 3).

$$n - \text{alkane ratio} = \frac{C_{31}+C_{33}}{C_{27}+C_{29}+C_{31}+C_{33}} \quad (3)$$

### 2.3 Climatic parameters

Climatic parameters, namely mean annual temperatures and precipitation, were correlated with the *n*-alkane ratio to test potential climatic influences on the *n*-alkane distribution. Generally, climate data for eastern Georgia are scarce because only few climate stations are located within the study area, and almost all stations show large data gaps after 1990 due to the political problems following the decay of the Soviet Union. Thus, reliable climate data cannot be derived from climate stations along the investigated transect or even for each sampled site. To overcome these limitations, we used mean annual temperature data of the climate station Tbilisi ([www.yr.no/place/Georgia/Tbilisi](http://www.yr.no/place/Georgia/Tbilisi)) that is located at 490 m a.s.l. and reports temperatures between 1962 and 1990. Based on this reference station, mean annual temperatures for our investigated sites were calculated under the assumption that temperature declines linearly with 0.65 K by 100 m increase in elevation (barometric formula of Schönwiese, 2008). The approximate altitude of each site was taken by hand-held GPS. For precipitation, we used an unpublished precipitation map of Georgia (V. Bagrationi Geographical Institute Tbilisi) that reports extrapolated mean annual precipitation between 1960 and 1990 (Fig. 2C). Since the available climate data average over ca. 30 years, only the *n*-alkane distributions from the topsoils representing averaged decadal signals (Wiesenberg et al., 2004) can robustly be correlated with the climate data. In contrast, the *n*-alkane distributions from the corresponding modern plants represent annual signals of the sampling year 2016, and can thus not robustly be correlated with the available climate data but will be shown for completeness.

### 3 Results

*n*-Alkanes are present in all analysed modern plant and topsoil samples from eastern Georgia, and the *n*-alkane and climate data are provided in the supporting online material SOM-3.

All samples show a distinct dominance of odd-numbered long-odd-chained *n*-alkanes (>C<sub>25</sub>). The total *n*-alkane concentrations (C<sub>25</sub>-C<sub>35</sub>) of the samples range from 1.1 to 299.1 µg g<sup>-1</sup> sediment/dry material, and modern plant samples have systematically higher *n*-alkane amounts/concentrations than topsoil samples (Fig. 3a). All samples show distinct odd-over-even predominance (OEP) values between 3.1 and 18.7 (Fig. 3b), and especially for samples from sites with grassland/herbs, modern plant material shows systematically higher OEP values than the corresponding topsoil material (Fig. 3b). Average chain-length (ACL) values range from 28.6 to 31.2, with samples from sites with grassland/herbs showing systematically higher values than those from sites with deciduous trees/shrubs (Fig. 3c). Systematic differences in chain-length patterns are further reported by the *n*-alkane ratio that ranges from 0.16 to 0.77 (Fig. 3d): Lower *n*-alkane ratios (<0.5) are characteristic for the samples from sites with deciduous trees/shrubs, whereas higher ratios (>0.5) are typical for samples from sites with grassland sites. Furthermore, systematic differences in the chain-length patterns between samples from sites with deciduous trees/shrubs and sites with grassland sites/herbs are illustrated in Fig. 4: Samples from sites with deciduous

~~sites/trees/shrubs~~ show a clear dominance of C<sub>29</sub>, whereas samples from ~~sites with~~ grassland ~~sites/herbs~~ are dominated by C<sub>31</sub>. This pattern is characteristic for both, analysed modern plant and topsoil material, although the standard deviation of C<sub>29</sub> and C<sub>31</sub> ~~is wider in the analysed of~~ modern plant samples ~~is generally wider, what holds especially true for sites with grassland/herbs~~ (Fig. 4a4).

- 5 ~~*n*-Alkanes from modern plants and topsoils show no correlation with mean annual temperatures for both, *n*-alkanes from sites with grassland/herbs ( $R^2 = 0.0873$  for modern plants;  $R^2 = 0.0947$  for topsoils) and from sites with deciduous trees/shrubs ( $R^2 = 0.0254$  for modern plants;  $R^2 = 0.1855$  for topsoils) (Fig. 5a). With respect to mean annual precipitation, no correlation exists for all *n*-alkanes from sites with grassland/herbs ( $R^2 = 0.0001$  for modern plants;  $R^2 = 0.0621$  for topsoils), and *n*-alkanes from topsoils from sites with deciduous trees/shrubs ( $R^2 = 0.0085$ ). Only the *n*-alkanes from modern plants from sites with deciduous~~
- 10 ~~trees/shrubs show a weak correlation with mean annual precipitation ( $R^2 = 0.3362$ ) (Fig. 5b).~~

## 4 Discussion

### 4.1 *n*-Alkane ~~pattern~~patterns in modern plants and topsoils

- All modern plant and topsoil samples from eastern Georgia show a distinct ~~pronounced OEP that indicate their good preservation and prove OEP. This proves~~ their leaf wax origin (Eglinton and Hamilton, 1967). ~~Higher~~Most fresh plant material
- 15 ~~shows higher~~ *n*-alkane concentrations and OEPs ~~are typical for fresh and non-degraded plant material, while the topsoils compared with topsoils show lower concentrations and OEPs~~ (Fig. 3a, b) ~~that might indicate enhanced~~, ~~which indicates ongoing degradation of organic matter (OM) in the topsoils~~ (Bugge et al., 2010; Schäfer et al., 2016b). ~~Although the degradation of organic matter (OM) already started~~However, also in the topsoils, the OEP values ~~in those samples~~ are mostly ~~not < 5~~ ~~which still~~. This indicates ~~astill~~ good preservation of the *n*-alkanes ~~and allows, allowing~~ the reconstruction of former vegetation
- 20 ~~patterns. Accordingly, both the ACL and the *n*-alkane ratio report distinct differences in the chain-length patterns for modern plant and topsoil samples from sites with deciduous trees/shrubs and sites with grassland~~ (Fig. 3, 4). ~~Higher values and a C<sub>31</sub>-dominance are characteristic for *n* alkanes from sites with grassland, whereas lower~~: Lower ACL values and a dominance of C<sub>29</sub> are characteristic for ~~samples from~~ sites with deciduous trees/shrubs, and higher ACL values and a C<sub>31</sub> dominance for ~~*n*-alkanes from sites with grassland/herbs~~ (Fig. 3c, d).
- 25 ~~However, modern plant samples 9p, 25p and 34p originating from sites with grassland show a chain length dominance of C<sub>29</sub>, whereas modern plant samples 3p and 29p and topsoil sample 23s originating from sites with deciduous trees are dominated by C<sub>31</sub> homologues. Thus, these samples do not support the proposed chain length patterns for the respective vegetation types. At the sampling site of topsoil sample 23s, hornbeam trees were only growing in patches and the site is and probably was intensively used for grazing activities. Thus, the dominance of C<sub>31</sub> in the topsoil of this site is most likely an inherited signal from former land use. Moreover, as turnover times of *n* alkanes range in order of decades (Wiesenberg et al., 2004), land use and vegetation changes might actually be a possible explanation for the observed contradictory *n* alkane pattern in the topsoil of this site. For modern plant samples it is possible that the described outliers reflect: (i) a large species-specific variability of~~
- 30



the *n*-alkane pattern that was repeatedly reported in the literature (Bush and McInerney, 2013; Diefendorf et al., 2011) and/or (ii) an influence of annual temperatures (Tipple and Pagani, 2013; Bush and McInerney, 2015; Sachse et al., 2006) and hydro-climatic conditions (Hoffmann et al., 2013) on the homologue patterns. For example, modern plant samples from site 9 and 34 were taken in high altitudes of the Kura fold-and-thrust belt (1659 m a.s.l.) and in the floodplain of the upper Alazani Basin (445 m a.s.l.), respectively. These sites represent either extreme temperatures (site 9) or enhanced water availability (site 34) that might have affected the *n*-alkane synthesis at these locations. As the respective topsoil samples from sites 9 and 34 averaging the *n*-alkane patterns over several years/decades and show the expected C<sub>31</sub>-dominance, modern plant samples probably record a more variable annual and/or monthly *n*-alkane pattern from these sites. This is also supported by the generally larger standard deviations of the *n*-alkane abundances from modern plant material taken at some of our studied sites.

Taken together, although it has recently been questioned whether *n*-alkane patterns originate from leaf waxes and are suitable to distinguish between different vegetation types at all (Bush and McInerney, 2013), our results from eastern Georgia confirm the leaf wax origin and chemotaxonomic discrimination power of the *n*-alkane pattern from modern plant and topsoil samples on a regional scale. Moreover, these results are in good agreement with previous transect studies from Central and South-Eastern Europe (Zech et al., 2009; Zech et al., 2013; Schäfer et al., 2016b). Thus, *n*-alkane patterns have a great potential to reconstruct paleovegetation when used in regional sedimentary archives.

However, modern plant samples 9p, 25p and 34p originating from sites with grassland/herbs show a chain-length dominance of C<sub>29</sub>, whereas modern plant samples 3p, 5p and 29p and topsoil sample 23s originating from sites with deciduous trees are dominated by C<sub>31</sub> homologues. Thus, these samples do not support the proposed chain-length patterns for the respective vegetation forms. At the sampling site of topsoil sample 23s, hornbeam trees were only growing in patches and the site is and probably was intensively used for grazing activities. Likewise, Holtvoeth et al. (2016) report similar *n*-alkane distributions from the Ohrid Basin, i.e. a dominance of grass-derived (C<sub>31</sub>) *n*-alkanes in the topsoil of a beech forest. Besides vegetation changes, they suggest that the *n*-alkanes from the grassy undergrowth overproportionally enter the soil, whereas the leaf litter is more mobile and might thus get relocated. Therefore, the dominance of C<sub>31</sub> in the topsoil of site 23 might be an inherited signal from former land-use/vegetation change, and/or because *n*-alkanes from the grassy undergrowth were overproportionally incorporated into the topsoil. For the observed outliers of the modern plant samples, it is possible that these reflect: (i) a relatively large species-specific variability of the produced *n*-alkane pattern that was repeatedly reported in the literature (Bush and McInerney, 2013; Diefendorf et al., 2011) and/or (ii) an influence of annual temperature (Tipple and Pagani, 2013; Bush and McInerney, 2015; Sachse et al., 2006) and moisture variations (Hoffmann et al., 2013) on the homologue patterns. Modern grassland/herb sample 9 with C<sub>29</sub> dominance was taken from a site in higher altitudes of the Kura-fold-and-thrust-belt (1659 m a.s.l.) that could potentially be affected by extreme temperatures. Therefore, this factor might potentially have affected the *n*-alkane synthesis at this site during the growing season of 2016. In contrast, modern grassland/herb samples 25 and 34 with C<sub>29</sub> dominance were taken from sites located at an inactive former floodplain of the Alazani River in the upper Alazani Basin at altitudes of 570 and 445 m a.s.l., respectively (Suchodoletz et al., in press). Thus, they are not expected to be affected by temperature or moisture extremes. Similarly, modern deciduous tree/shrub samples 3 (924 m a.s.l.) and 5 (830 m a.s.l.) with a

strong contribution of C<sub>31</sub>, and modern deciduous tree/shrub sample 29 (774 m a.s.l.) with a dominance of C<sub>31</sub> were not taken from sites where an influence of temperature or moisture extremes should be expected. Therefore, given that the respective topsoil samples from these sites that average the *n*-alkane patterns over several years/decades (Wiesenberg et al., 2004) show the expected C<sub>31</sub> dominance for grassland sites 9, 25 and 34, and the expected C<sub>29</sub> dominance for deciduous tree/shrub sites 3, 5 and 29, an influence of interannual temperature or moisture variations on the observed outliers of the *n*-alkane patterns in 2016 can only be assumed so far. A generally higher sensitivity of our modern plant samples towards interannual variations of the *n*-alkane synthesis is also supported by generally larger standard deviations of the *n*-alkane parameters from modern plant material (Figs. 3 and 4).

To get more insight into possible climatic influences on the *n*-alkane distributions in eastern Georgia, we correlated our *n*-alkane ratios with mean annual temperatures and precipitation (Fig. 5). The only available climate information for our investigated transect are averaged temperature and precipitation data for the timespan between 1960 and 1990. These can only be correlated with the *n*-alkane signals from topsoils, since these are averaged over a similar decadal timescale (Wiesenberg et al., 2004). As shown in Fig. 5, the *n*-alkanes from topsoils do neither correlate with mean annual temperatures nor with precipitation. The weak correlation between the *n*-alkane pattern of modern plants from sites with deciduous trees/shrubs for the sampling year 2016 and mean annual precipitation between 1960 and 1990 (Fig. 5b) should not be over-interpreted due to the different periods that are covered by both datasets. Summing up, a significant influence of climatic factors on the *n*-alkane patterns in eastern Georgia could not be observed. These findings seem to contradict former studies that repeatedly found correlations between the *n*-alkane distribution and temperature (Tipple et al., 2013; Bush and McInerney, 2015) or precipitation (Hoffmann et al., 2013). However, those studies investigated climatic transects with wide temperature gradients of >20 °C and precipitation gradients spanning ~2000 mm of annual rainfall. In contrast, our investigated transect shows much smaller gradients in temperature and precipitation of 7.9 °C and ca. 1400 mm/a, respectively. Therefore, climate has obviously no significant influence on the *n*-alkane distribution in topsoils of eastern Georgia. Since climate seems a negligible factor for the *n*-alkane distribution in topsoils, this further justifies our approach to reconstruct regional paleovegetation based on *n*-alkane patterns at least in eastern Georgia.

Taken together, although it has recently been questioned whether *n*-alkane patterns originate from leaf waxes and are suitable to distinguish between different vegetation forms at all (Bush and McInerney, 2013), our results from eastern Georgia confirm the leaf wax origin and chemotaxonomic discrimination power of the *n*-alkane pattern from modern plant and topsoil samples for this region. This agrees with previous studies from South-Eastern Europe where C<sub>29</sub> was reported as the most abundant homologue derived from deciduous trees and shrubs, and C<sub>31</sub> as mainly derived from grasses and herbs (Zech et al., 2009; Holtvoeth et al., 2016). A similar good chemotaxonomic discrimination power of the *n*-alkane pattern has also been reported from other regions, although slight differences in the patterns were observed between the individual regions: Schäfer et al. (2016b) found that high amounts of C<sub>31</sub> and C<sub>33</sub> were produced at grassland/herb sites in temperate Central Europe, and that sites with deciduous forests/shrubs were mostly dominated by C<sub>27</sub>. In tropical ecosystems, several studies observed C<sub>29</sub> as the most dominant *n*-alkane homologue in C3 rainforest trees, whereas C4 grasses and herbs mainly produced C<sub>33</sub> as the most

abundant homologue (Feakins et al., 2016; Vogts et al., 2009; Garcin et al., 2014). For more arid South-Africa, a dominance of  $C_{31}$  and  $C_{33}$  *n*-alkanes is reported for succulent plants and regional soils (Carr et al., 2014). Altogether, most regional studies show a good chemotaxonomic discrimination power of *n*-alkane patterns, demonstrating their great potential to reconstruct paleovegetation when used in sedimentary archives at least in these regions.

## 5 4.2 Implications for reconstructing paleovegetation ~~compositions~~composition

When using leaf wax *n* alkanes in sedimentary archives, degradation of OM needs to be taken into account. Ongoing degradation leads to preferential losses of the most abundant homologues. Thus, changes of the homologue distribution and potentially confounding degradation effects can be visualized in an end member model (Zech et al., 2009; Zech et al., 2013; Schäfer et al., 2016b) that is based on litter and topsoil samples from deciduous forests and grasslands in Europe. As illustrated in Fig. 5, our end member model uses the normalized *n* alkane ratio  $(C_{31}+C_{33})/(C_{27}+C_{29}+C_{31}+C_{33})$  on the y-axis and the OEP on the x-axis. While the *n* alkane ratio differentiates between vegetation with higher values for grasses and grassland soils and lower values for deciduous trees and deciduous forest soils, the OEP is used as a proxy for degradation. The respective trendlines, referred to as “degradation lines” for grass vegetation and deciduous trees, illustrate how the *n* alkane ratio changes with degradation (Fig. 5). With increasing degradation, the degradation lines of grasses and deciduous trees converge and the discrimination power of the *n* alkane ratio becomes less. The study of Schäfer et al. (2016b) uses a slightly different *n* alkane ratio that excluded  $C_{29}$  and improved the discrimination power for their region. However, this is not the case for eastern Georgia where  $C_{29}$  and  $C_{31}$  are the most abundant homologues in our samples.

Our *n* alkane samples from modern plants and topsoils from eastern Georgia show clear differences between sites with deciduous trees and those with grassland, and plot along the respective degradation lines of grasses and deciduous trees in the endmember plot (see Fig. 5). Thus, they are in good agreement with the reference samples from South Eastern Europe that were presented in the end member model of Zech et al. (2013). Accordingly, we integrated our *n* alkane results from eastern Georgia into that dataset and recalculated the degradation lines. As mentioned above and illustrated in Fig. 5, some outliers of modern plants and topsoils from sites with deciduous trees plot in the direction of the grass degradation line and vice versa. These outliers can possibly be explained by mixed vegetation and anthropogenic land use at the specific sites as well as temperature and hydroclimatic effects (see chapter 4.1). Thus, these samples are not exemplary and were not considered when adjusting the degradation lines.

The adjusted degradation lines can now be used to calculate the relative vegetation contributions of grasses and deciduous trees in paleosamples that were taken from sedimentary archives from the southern Caucasus and comparable regions. Depending on the vegetation type that contributed most to the sample, the *n* alkanes will plot in the two component mixing end member model on or near to one of the degradation lines of grasses or deciduous trees, respectively. The percentage of grass can be calculated by the following formula:

When using leaf wax *n*-alkanes in sedimentary archives, degradation of OM needs to be taken into account since this process leads to preferential losses of the most abundant homologues. Changes of the homologue distribution and potentially confounding degradation effects can be visualized in an end-member model (Zech et al., 2009; Zech et al., 2013) that is based on modern plant/litter and topsoil samples from sites with deciduous trees/shrubs and sites with grassland/herbs in Central and South-Eastern Europe. This model uses the normalized *n*-alkane ratio  $(C_{31}+C_{33})/(C_{27}+C_{29}+C_{31}+C_{33})$  on the y-axis and the OEP on the x-axis: Whereas the *n*-alkane ratio discriminates between deciduous trees/shrubs and deciduous forest soils that show lower values on the one hand, and grasses/herbs and soils from grassland/herb sites that show higher values on the other hand, the OEP is used as a proxy for degradation. The respective trend lines, referred to as “degradation lines” for grass/herb and deciduous tree/shrub vegetation, illustrate how the *n*-alkane ratio changes with degradation (Fig. 6): With increasing degradation, the degradation lines of grasses and deciduous trees converge, and therefore the discrimination power of the *n*-alkane ratio systematically reduces with increasing degradation. Given that most modern plant and topsoil *n*-alkanes from eastern Georgia discriminate well between sites with deciduous trees/shrubs and sites with grassland/herbs when using the *n*-alkane ratio of Zech et al. (2013; Eq. 3), we integrated our *n*-alkane samples from eastern Georgia into that end member model to enhance the data base and thus to better back the model for eastern Georgia (Fig 6): Whereas most samples from eastern Georgia plot well along the respective degradation lines of grasses/herbs and deciduous trees/shrubs, this is not the case for modern plant samples 3p, 5p, 9p, 25p, 29p and 34p and topsoil sample 23s. For the latter sample this was possibly caused by former land use/vegetation changes and/or overproportional incorporation of grass-derived *n*-alkanes into the topsoil, and for plant sample 9p possibly by strong interannual temperature variations, whereas for the remaining samples the causes still remain unclear (Fig. 6, see chapter 4.1). All these samples were regarded as outliers and thus not considered when recalculating the degradation lines after integration of the samples from eastern Georgia. Given that all but one topsoil sample that average the *n*-alkane signal over several decades show a very good discriminative power between grass/herb and deciduous tree/shrub vegetation, this demonstrates the robustness of the *n*-alkane signal as a proxy for paleovegetation reconstructions at least in eastern Georgia (Fig. 6). The recalculated degradation lines for the relative contributions of grasses/herbs and deciduous trees/shrubs can now serve as a base to calculate former vegetation distributions from paleosamples that were taken from sedimentary archives in eastern Georgia and neighbouring regions of the central southern Caucasus: Depending on the vegetation form that contributed most to the OM of a sample, the *n*-alkanes will plot in the two-component mixing end-member model on or near to one of the degradation lines of grasses or deciduous trees, respectively. The OEP lowers with increasing degradation. Therefore, increasing degradation reduces the accuracy of the correction, since the degradation lines converge with lowered OEPs. The percentage of grasses/herbs can be calculated using the following formula:

$$\% \text{ grass} = \frac{n\text{-alkane ratio (sample)} - \text{equation (degradation line trees)}}{\text{equation (degradation lines grass)} - \text{equation (degradation lines trees)}} * 100 \quad (4)$$

In principle, the accuracy of the correction is limited by the degradation state of a sample, i.e. it decreases with low OEPs and thus with converging degradation lines. Furthermore, numerical corrections can yield even negative or >100 %grass values, however the grass percentages should not be over interpreted and instead be considered as semi quantitative estimates. This

effect is caused by the large scatter in the reference sample data set that can only be reduced towards more realistic values by integrating more regional reference samples into the end-member model as we did during our study.

Nevertheless, for more robust interpretations of *n*-alkane patterns (i.e. the ACL or ratios) in paleoapplications, they should be checked and corrected for degradation effects with regionally derived end-members. Although the OEP should generally decrease when plants are degraded and incorporated into soil organic matter, samples 16p/16s, 20p/20s, 23p/23s and 35p/35s originating from sites with deciduous trees/shrubs do not show the expected pattern (Fig. 6). However, the differences in the OEP values from those sites are only minor (i.e. <1) and thus negligible, but the degradation of leaf wax *n*-alkanes in soils and sediments is not fully understood so far.

The observed scattering of the individual samples around the degradation lines, potentially leading to negative or >100% values when calculating grass/herb percentages, could be caused by species-specific and environmental variability: Even within one species, environmental stress like different temperatures, moisture conditions, radiation levels, shading or soil nutrient availability can cause some variation of the *n*-alkane synthesis (Shepherd and Wynne Griffiths, 2006). Given that our *n*-alkane topsoil samples do not show correlations with temperature and annual precipitation (see chapter 4.1; Fig. 5), these factors can possibly be ruled out as causes for the observed scatter of topsoil *n*-alkanes in eastern Georgia.

## 5 Conclusions

This study systematically investigates long-chain *n*-alkanes in modern plant and topsoil material from eastern Georgia in the central southern Caucasus to test their potential for paleoenvironmental/paleovegetational studies. Our results illustrate that:

i.) Long-chain *n*-alkanes (C<sub>25</sub>-C<sub>35</sub>) show a clear OEP and thus originate from the leaf waxes of higher terrestrial plants and can thus serve as a valuable proxy that directly reflects the local vegetation.

ii.) The regional leaf wax *n*-alkane patterns show distinct and consistent differences between samples from sites with grassland/herbs and those sites with deciduous trees in eastern Georgia/shrubs: Samples from grassland/herb sites are characterized by a dominance of the homologue C<sub>31</sub>, whereas samples from sites with deciduous trees/shrubs show higher abundances of C<sub>29</sub>. Thus, leaf wax *n*-alkanes allow to differentiate that discriminate between these two vegetation types and forms have a great potential for paleovegetation reconstructions in the semi-humid to semi-arid central southern Caucasus region. However, besides vegetation changes, also other factors such as interannual temperature and moisture variability obviously influenced the chain-length abundances might also be influenced by species-specific variability of some modern plant samples, and environmental parameters, but these are factors such as land use/vegetation change or varying incorporation of OM from different plant types the chain-length abundances of one topsoil sample. These were only second-order effects at our investigated sites and accordingly, the *n*-alkane distributions of the topsoils did not show correlations with the climatic parameters annual temperature and precipitation along the investigated transect.

iii.) The degradation state of the *n*-alkanes has to be accounted for, given that increasing degradation reduces the vegetation-specific differences in the *n*-alkane homologue patterns. Thus, the degradation state of the *n*-

alkanes has to be taken into account. Therefore, to allow for a better correction of regional paleovegetation reconstructions from paleosamples we suggest ~~an updated~~ to integrate our plant and topsoil samples from eastern Georgia into the end-member model of Zech et al. (2013) that corrects for these effects. Generally, in case that the *n*-alkane patterns are very similar to those that were used for the original endmember model of Zech et al. (2013) as is the case for eastern Georgia, the accuracy of the correction can be increased by including our plant and topsoil samples from eastern Georgia, and allows for a better correction of regional paleosamples integrating more regional reference samples into the model. However, in case of *n*-alkane patterns that significantly deviate from those that were used for the endmember model of Zech et al (2013), the construction of an independent regional endmember model would be necessary.

Overall, our findings are in good agreement with other regional studies from Central and South-Eastern Europe and ~~show~~demonstrate the high potential of leaf wax *n*-alkanes for ~~paleoenvironmental research applications~~paleovegetation reconstruction. However, regional calibration studies with modern reference samples are a necessary base for the robust interpretation of leaf wax--derived long-chain *n*-alkanes in terms of former vegetation changes.

## 6 Data availability

The dataset that is used in this study is available via the supporting online material.

## Acknowledgements

We thank Ulrich Göres (Dresden) and Giorgi Merebashvili (Tbilisi) for their help during fieldwork. We thank Tobias Stalder (Bern) for laboratory work. This project was financially supported by the Swiss National Science Foundation (PP00P2-150590).

## 20 References

Buggle, B., Wiesenberg, G. L., and Glaser, B.: Is there a possibility to correct fossil *n*-alkane data for postsedimentary alteration effects?, *Applied Geochemistry*, 25, 947–957, doi:10.1016/j.apgeochem.2010.04.003, 2010.

Bush, R. T. and McInerney, F. A.: Leaf wax *n*-alkane distributions in and across modern plants: Implications for paleoecology and chemotaxonomy, *Geochimica et Cosmochimica Acta*, 117, 161–179, doi:10.1016/j.gca.2013.04.016, 2013.

Bush, R. T. and McInerney, F. A.: Influence of temperature and C4 abundance on *n*-alkane chain length distributions across the central USA, *Organic Geochemistry*, 79, 65–73, doi:10.1016/j.orggeochem.2014.12.003, 2015.

Carr, A. S., Boom, A., Grimes, H. L., Chase, B. M., Meadows, M. E., and Harris, A.: Leaf wax *n*-alkane distributions in arid zone South African flora: Environmental controls, chemotaxonomy and palaeoecological implications, *Organic Geochemistry*, 67, 72–84, doi:10.1016/j.orggeochem.2013.12.004, 2014.

- Connor, S. E. and Kvavadze, E. V.: Modelling late Quaternary changes in plant distribution, vegetation and climate using pollen data from Georgia, Caucasus, *Journal of Biogeography*, 36, 529–545, doi:10.1111/j.1365-2699.2008.02019.x, 2008.
- Connor, S. E., Thomas, I., Kvavadze, E. V., Arabuli, G. J., Avakov, G. S., and Sagona, A.: A survey of modern pollen and vegetation along an altitudinal transect in southern Georgia, Caucasus region, *Review of Palaeobotany and Palynology*, 5 129, 229–250, doi:10.1016/j.revpalbo.2004.02.003, 2004.
- Diefendorf, A. F., Freeman, K. H., Wing, S. L., and Graham, H. V.: Production of n-alkyl lipids in living plants and implications for the geologic past, *Geochimica et Cosmochimica Acta*, 75, 7472–7485, doi:10.1016/j.gca.2011.09.028, 2011.
- Egeland, C. P., Gasparian, B., Fadem, C. M., Nahapetyan, S., Arakelyan, D., and Nicholson, C. M.: Bagratashen 1, a stratified open-air Middle Paleolithic site in the Debed river valley of northeastern Armenia: A preliminary report, *Archaeological Research in Asia*, 8, 1–20, doi:10.1016/j.ara.2016.10.001, 2016.
- Eglinton, G. and Hamilton, R. J.: Leaf Epicuticular Waxes, *Science*, 156, 1322–1335, doi:10.1126/science.156.3780.1322, 1967.
- Eglinton, G., Hamilton, R. J., Raphael, R. A., and Gonzalez, A. G.: Hydrocarbon Constituents of the Wax Coatings of Plant 15 Leaves: A Taxonomic Survey, *Nature*, 193, 739–742, doi:10.1038/193739a0, 1962.
- Eglinton, T. I. and Eglinton, G.: Molecular proxies for paleoclimatology, *Earth and Planetary Science Letters*, 275, 1–16, doi:10.1016/j.epsl.2008.07.012, 2008.
- Feakins, S. J., Peters, T., Wu, M. S., Shenkin, A., Salinas, N., Girardin, C. A., Bentley, L. P., Blonder, B., Enquist, B. J., Martin, R. E., Asner, G. P., and Malhi, Y.: Production of leaf wax n-alkanes across a tropical forest elevation transect, *Organic Geochemistry*, 100, 89–100, doi:10.1016/j.orggeochem.2016.07.004, 2016.
- 20 Forte, A. M., Cowgill, E., Bernardin, T., Kreylos, O., and Hamann, B.: Late Cenozoic deformation of the Kura fold-thrust belt, southern Greater Caucasus, *Geological Society of America Bulletin*, 122, 465–486, doi:10.1130/B26464.1, 2010.
- Garcin, Y., Schefuß, E., Schwab, V. F., Garreta, V., Gleixner, G., Vincens, A., Todou, G., Séné, O., Onana, J.-M., Achoundong, G., and Sachse, D.: Reconstructing C 3 and C 4 vegetation cover using n -alkane carbon isotope ratios in recent lake sediments from Cameroon, Western Central Africa, *Geochimica et Cosmochimica Acta*, 142, 482–500, doi:10.1016/j.gca.2014.07.004, 2014.
- 25 Hoefs, M. J., Rijpstra, W. C., and Sinninghe Damsté, J. S.: The influence of oxic degradation on the sedimentary biomarker record I: Evidence from Madeira Abyssal Plain turbidites, *Geochimica et Cosmochimica Acta*, 66, 2719–2735, doi:10.1016/S0016-7037(02)00864-5, 2002.
- 30 Hoffmann, B., Kahmen, A., Cernusak, L. A., Arndt, S. K., and Sachse, D.: Abundance and distribution of leaf wax n-alkanes in leaves of Acacia and Eucalyptus trees along a strong humidity gradient in northern Australia, *Organic Geochemistry*, 62, 62–67, doi:10.1016/j.orggeochem.2013.07.003, 2013.



Holtvoeth, J., Rushworth, D., Copsey, H., Imeri, A., Cara, M., Vogel, H., Wagner, T., and Wolff, G. A.: Improved end-member characterisation of modern organic matter pools in the Ohrid Basin (Albania, Macedonia) and evaluation of new palaeoenvironmental proxies, *Biogeosciences*, 13, 795–816, doi:10.5194/bg-13-795-2016, 2016.

- Joannin, S., Ali, A., Ollivier, V., Roiron, P., Peyron, O., Chevaux, S., Nahapetyan, S., Tozalakyan, P., Karakhanyan, A., and Chataigner, C.: Vegetation, fire and climate history of the Lesser Caucasus: A new Holocene record from Zarishat fen (Armenia), *J. Quaternary Sci.*, 29, 70–82, doi:10.1002/jqs.2679, 2014.
- Kolattukudy, P. E. and Walton, T. J.: The biochemistry of plant cuticular lipids, *Progress in the Chemistry of Fats and other Lipids*, 13, 119–175, doi:10.1016/0079-6832(73)90006-2, 1973.
- Liu, W. and Huang, Y.: Compound specific D/H ratios and molecular distributions of higher plant leaf waxes as novel paleoenvironmental indicators in the Chinese Loess Plateau, *Organic Geochemistry*, 36, 851–860, doi:10.1016/j.orggeochem.2005.01.006, 2005.
- Lydolph, P. E. (Ed.): *Climates of the Soviet Union: [138 Tab.], World survey of climatology*, 7, Elsevier, Amsterdam, 443 pp., 1977.
- Marseille, F., Disnar, J. R., Guillet, B., and Noack, Y.: n-Alkanes and free fatty acids in humus and A1 horizons of soils under beech, spruce and grass in the Massif-Central (Mont-Lozère), France, *European Journal of Soil Science*, 50, 433–441, doi:10.1046/j.1365-2389.1999.00243.x, 1999.
- Messenger, E., Belmecheri, S., Grafenstein, U. von, Nomade, S., Ollivier, V., Voinchet, P., Puaud, S., Courtin-Nomade, A., Guillou, H., Mgeladze, A., Dumoulin, J.-P., Mazuy, A., and Lordkipanidze, D.: Late Quaternary record of the vegetation and catchment-related changes from Lake Paravani (Javakheti, South Caucasus), *Quaternary Science Reviews*, 77, 125–140, doi:10.1016/j.quascirev.2013.07.011, 2013.
- Poynter, J. G., Farrimond, P., Robinson, N., and Eglinton, G.: Aeolian-Derived Higher Plant Lipids in the Marine Sedimentary Record: Links with Palaeoclimate, in: *Paleoclimatology and Paleometeorology: Modern and Past Patterns of Global Atmospheric Transport*, Leinen, M., and Sarnthein, M. (Eds.), Springer Netherlands, Dordrecht, 435–462, 1989.
- Sachse, D., Radke, J., and Gleixner, G.:  $\delta D$  values of individual n-alkanes from terrestrial plants along a climatic gradient – Implications for the sedimentary biomarker record, *Organic Geochemistry*, 37, 469–483, doi:10.1016/j.orggeochem.2005.12.003, 2006.
- Sagheb-Talebi, K., Sajedi, T., and Pourhashemi, M.: *Forests of Iran: A treasure from the past, a hope for the future*, Plant and Vegetation, 10, Springer Netherlands, Dordrecht, s.l., 55 pp., 2014.
- Schäfer, I. K., Bliedtner, M., Wolf, D., Faust, D., and Zech, R.: Evidence for humid conditions during the last glacial from leaf wax patterns in the loess–paleosol sequence El Paraíso, Central Spain, *Quaternary International*, doi:10.1016/j.quaint.2016.01.061, 2016a.
- Schäfer, I. K., Lanny, V., Franke, J., Eglinton, T. I., Zech, M., Vysloužilová, B., and Zech, R.: Leaf waxes in litter and topsoils along a European transect, *SOIL*, 2, 551–564, doi:10.5194/soil-2-551-2016, 2016b.



- Schatz, A.-K., Zech, M., Buggle, B., Gulyás, S., Hambach, U., Marković, S. B., Sümegi, P., and Scholten, T.: The late Quaternary loess record of Tokaj, Hungary: Reconstructing palaeoenvironment, vegetation and climate using stable C and N isotopes and biomarkers, *Quaternary International*, 240, 52–61, doi:10.1016/j.quaint.2010.10.009, 2011.
- Schefuß, E., Schouten, S., Jansen, J. H. F., and Sinninghe Damsté, J. S.: African vegetation controlled by tropical sea surface temperatures in the mid-Pleistocene period, *Nature*, 422, 418–421, doi:10.1038/nature01500, 2003.
- Schönwiese, C.-D.: *Klimatologie*, 1. Aufl., UTB M, UTB GmbH, Stuttgart, 2008.
- Schwark, L., Zink, K., and Lechterbeck, J.: Reconstruction of postglacial to early Holocene vegetation history in terrestrial Central Europe via cuticular lipid biomarkers and pollen records from lake sediments, *Geol*, 30, 463, doi:10.1130/0091-7613(2002)030<0463:ROPTEH>2.0.CO;2, 2002.
- 10 [Shepherd, T. and Wynne Griffiths, D.: The effects of stress on plant cuticular waxes. \*The New phytologist\*, 171, 469–499, doi:10.1111/j.1469-8137.2006.01826.x, 2006.](#)
- Suchodoletz, H. von, Menz, M., Kühn, P., Sukhishvili, L., and Faust, D.: Fluvial sediments of the Algeti River in southeastern Georgia — An archive of Late Quaternary landscape activity and stability in the Transcaucasian region, *Catena*, 130, 95–107, doi:10.1016/j.catena.2014.06.019, 2015.
- 15 Tipple, B. J. and Pagani, M.: Environmental control on eastern broadleaf forest species' leaf wax distributions and D/H ratios, *Geochimica et Cosmochimica Acta*, 111, 64–77, doi:10.1016/j.gca.2012.10.042, 2013.
- [Vogts, A., Moossen, H., Rommerskirchen, F., and Rullkötter, J.: Distribution patterns and stable carbon isotopic composition of alkanes and alkan-1-ols from plant waxes of African rain forest and savanna C3 species, \*Organic Geochemistry\*, 40, 1037–1054, doi:10.1016/j.orggeochem.2009.07.011, 2009.](#)
- 20 Wiesenberg, G. L., Schwarzbauer, J., Schmidt, M. W., and Schwark, L.: Source and turnover of organic matter in agricultural soils derived from n-alkane/n-carboxylic acid compositions and C-isotope signatures, *Organic Geochemistry*, 35, 1371–1393, doi:10.1016/j.orggeochem.2004.03.009, 2004.
- Wolf, D., Baumgart, P., Meszner, S., Fülling, A., Haubold, F., Sahakyan, L., Meliksetian, K., and Faust, D.: Loess in Armenia – stratigraphic findings and palaeoenvironmental indications, *Proceedings of the Geologists' Association*, 127, 29–
- 25 39, doi:10.1016/j.pgeola.2016.02.002, 2016.
- Zech, M., Buggle, B., Leiber, K., Marković, S., Glaser, B., Hambach, U., Huwe, B., Stevens, T., Sümegi, P., Wiesenberg, G., and Zöller, L.: Reconstructing Quaternary vegetation history in the Carpathian Basin, SE-Europe, using n-alkane biomarkers as molecular fossils, *E&G – Quaternary Science Journal*; Vol. 58 No 2; 148-155; ISSN 0424-7116, doi:10.3285/eg.58.2.03, 2009.
- 30 Zech, R., Zech, M., Marković, S., Hambach, U., and Huang, Y.: Humid glacial, arid interglacials?: Critical thoughts on pedogenesis and paleoclimate based on multi-proxy analyses of the loess–paleosol sequence Crvenka, Northern Serbia, *Palaeogeography, Palaeoclimatology, Palaeoecology*, 387, 165–175, doi:10.1016/j.palaeo.2013.07.023, 2013.

Zhang, Z., Zhao, M., Eglinton, G., Lu, H., and Huang, C.: Leaf wax lipids as paleovegetational and paleoenvironmental proxies for the Chinese Loess Plateau over the last 170kyr, *Quaternary Science Reviews*, 25, 575–594, doi:10.1016/j.quascirev.2005.03.009, 2006.



Figure 1: Overview of the Caucasus Region. The red rectangle marks the study area.



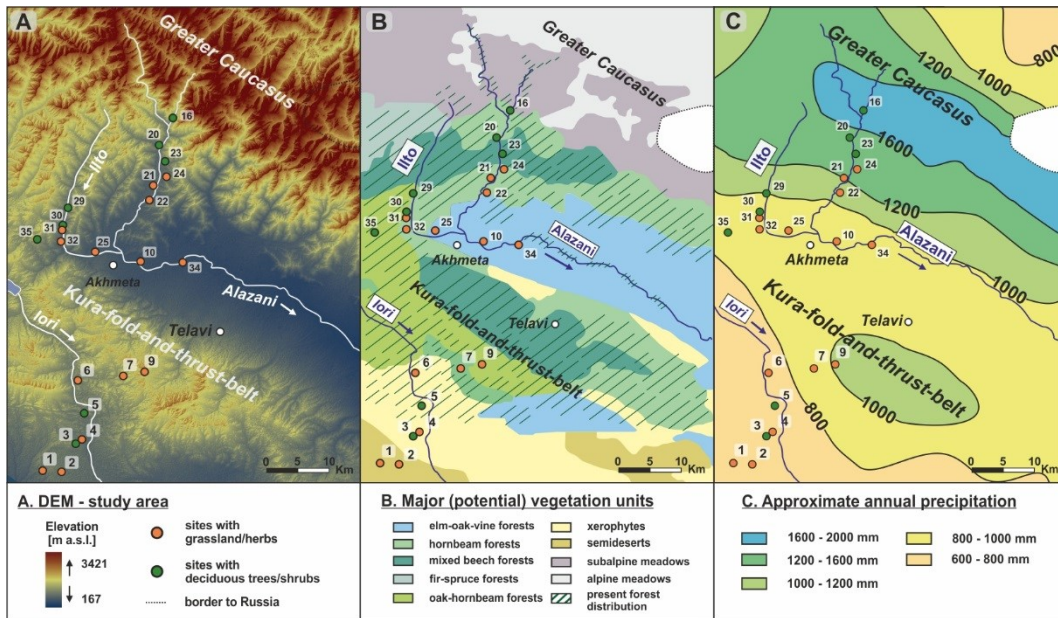
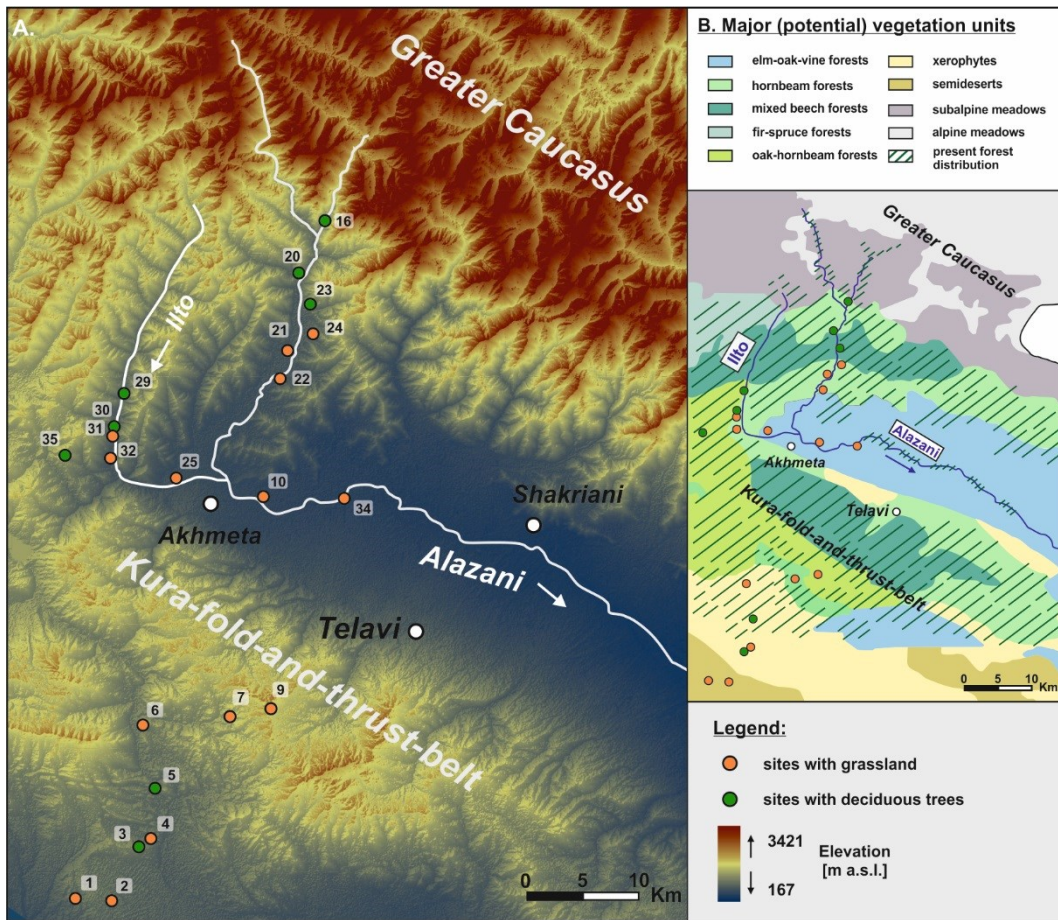
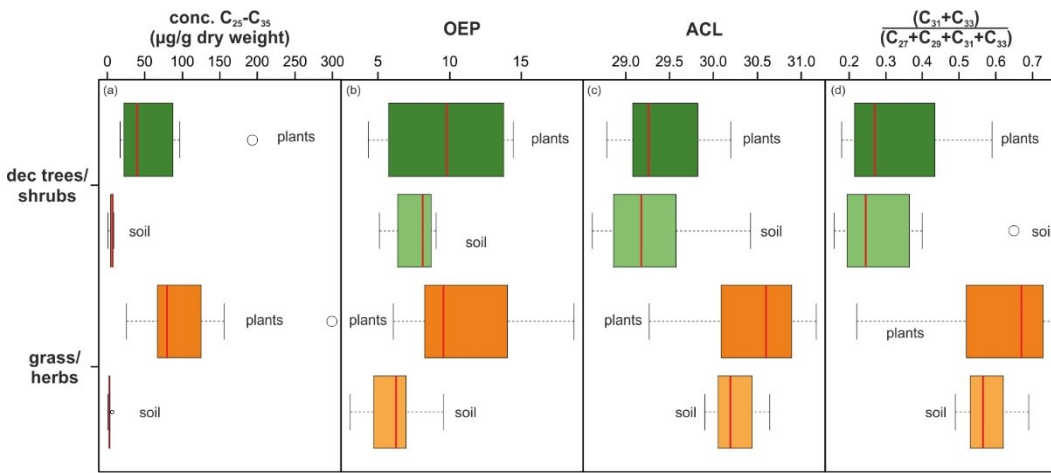
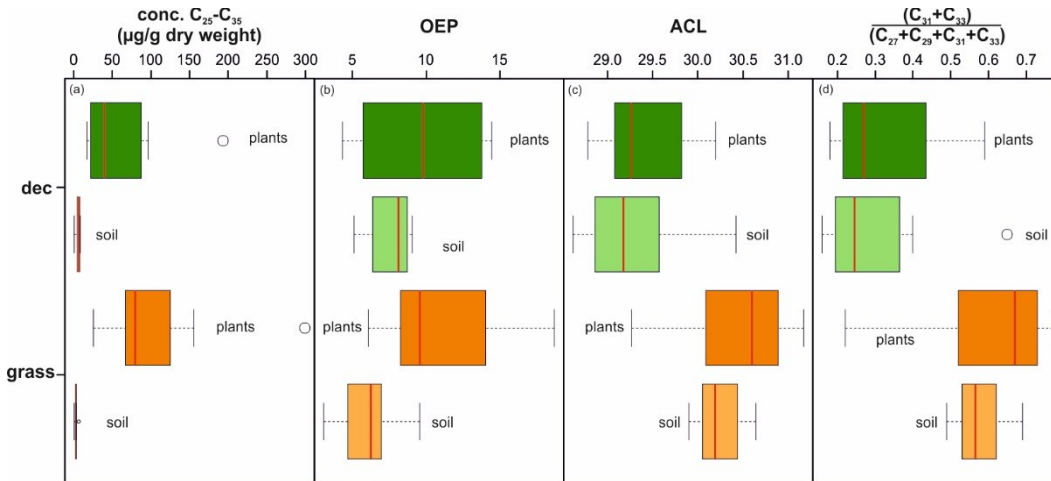


Figure 2: A. Digital elevation model of the study area (Aster DEM). Locations of plant and topsoil samples are indicated by an orange dot for sites with grassland/herbs, and a green dot for sites with deciduous trees/shrubs. B. Current natural potential vegetation in the study area (based on the bioclimatic map of Connor & Kvavadze 2008). The current distribution of forests in the study area (second half of 20th century) is derived from Soviet military maps 1:200,000. C. Approximate annual precipitation in the study area (based on unpublished precipitation map of the V. Bagrationi Geographical Institute Tbilisi that averages precipitation data between 1960 and 1990).

5



10

Figure 3: Box plots of *n*-alkanes from modern plant and topsoil samples. (a) Concentration  $\mu\text{g/g}$  ( $C_{25}\text{-}C_{35}$ ), (b) Odd-over-even predominance (OEP), (c) Average chain length (ACL), (d) *n*-alkane ratio  $(C_{31}+C_{33})/(C_{27}+C_{29}+C_{31}+C_{33})$ . Dec = sites with deciduous sites/trees/shrubs ( $n = 16$ ); grass = sites with grassland-sites/herbs ( $n = 28$ ). ~~Box~~ The box plots show median (red line), interquartile range (IQR) with upper (75 %) and lower (25 %) quartiles. (full rectangles) and outliers (dashed lines).

15

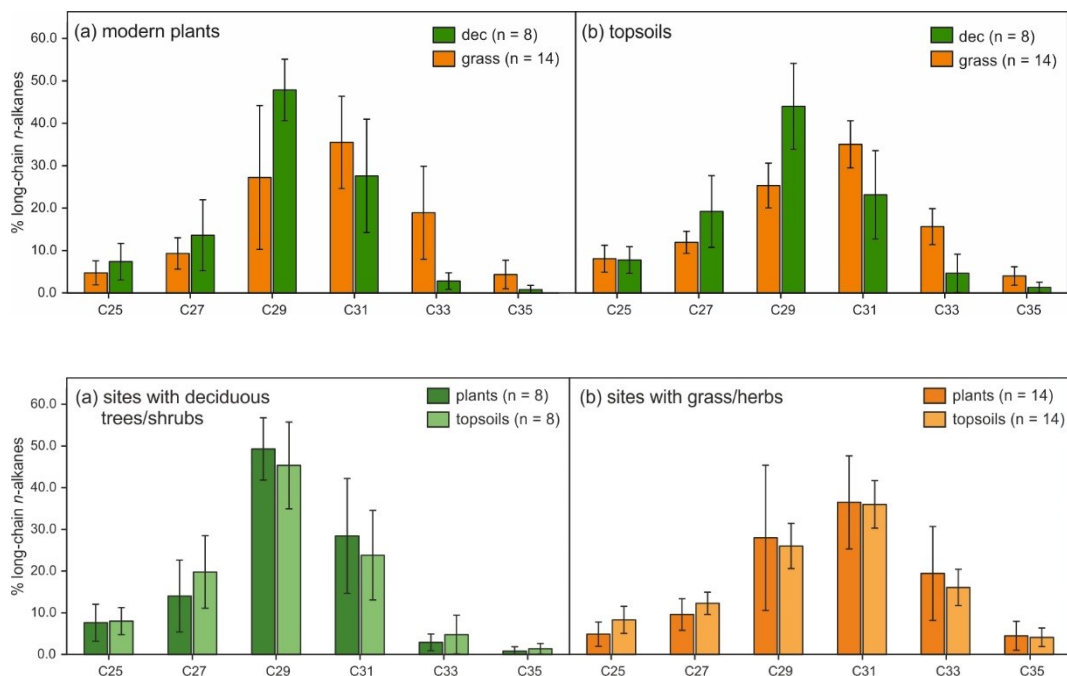


Figure 4: Chain length distribution patterns for long-chain *n*-alkanes in modern plants and topsoils from sites with (a) deciduous trees/shrubs, and (b) grassland/herbs.

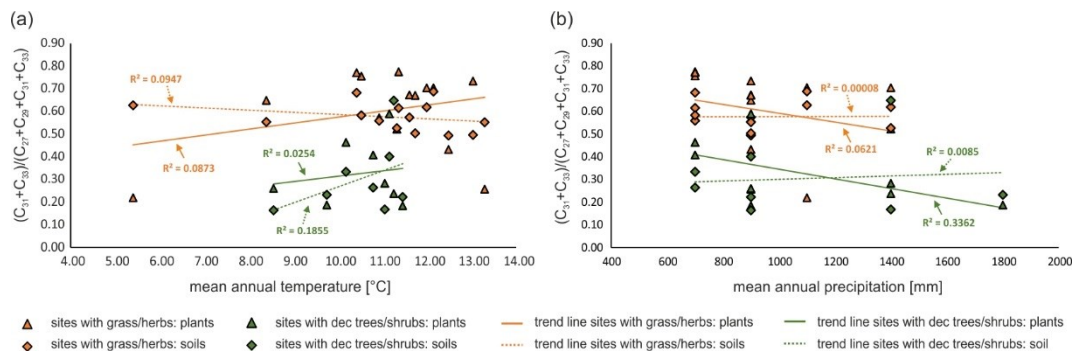
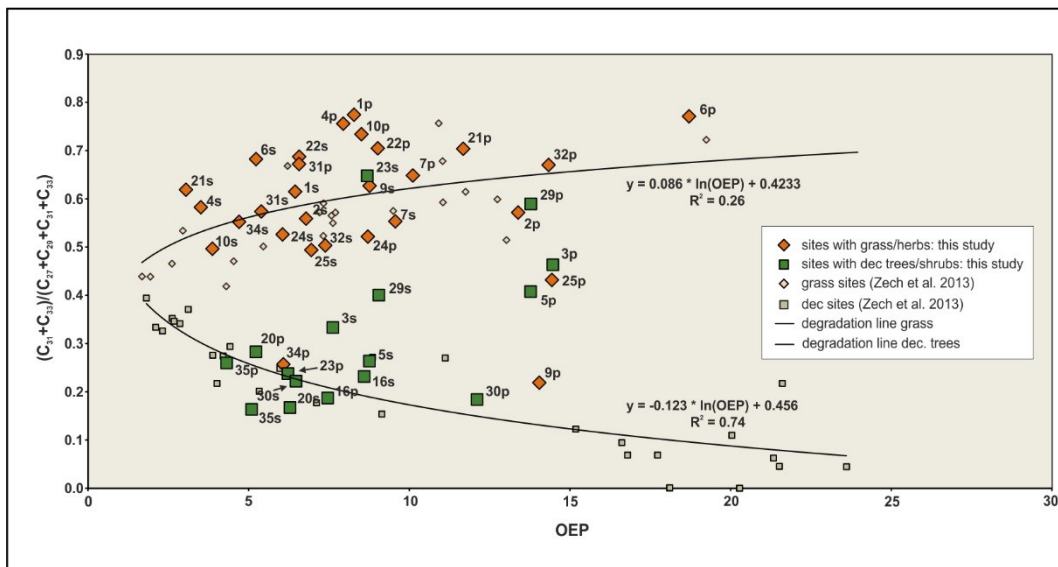
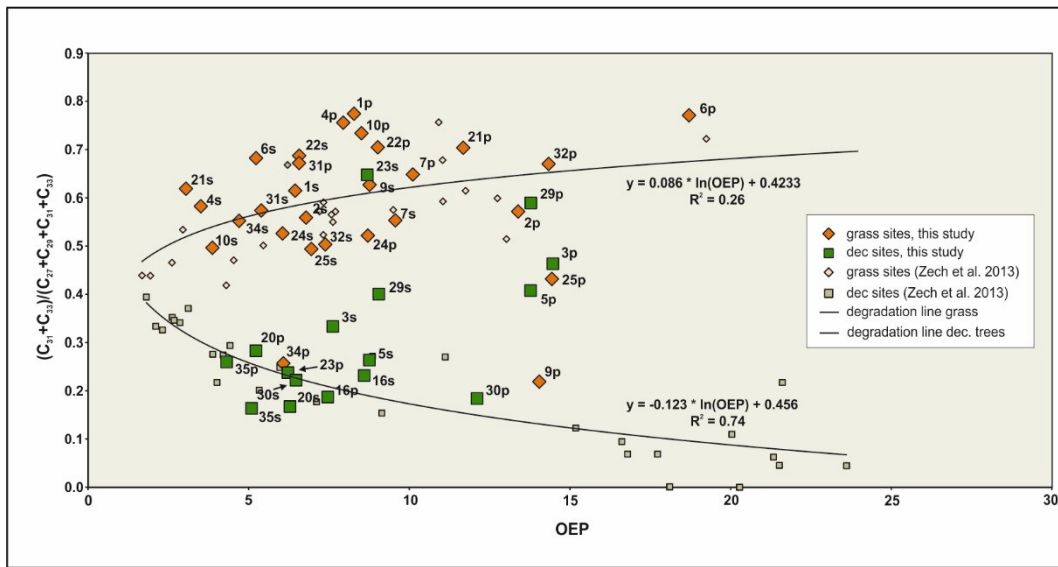


Figure 5: *n*-Alkane ratios from modern plant and topsoil samples plotted against (a) mean annual temperatures and (b) mean annual precipitation. Dotted trend lines are linear regression lines for *n*-alkanes of modern plants from sites with grassland in (a) modern plants and (b) topsoils-/herbs (orange) and sites with deciduous trees/shrubs (green), whereas solid trend lines refer to *n*-alkanes from topsoils.



5 Figure 56: End-member plot for the *n*-alkane ratio  $(C_{31}+C_{33})/(C_{27}+C_{29}+C_{31}+C_{33})$ . *n*-Alkane/alkane results from plant and topsoil samples from the upper Alazani catchment eastern Georgia were integrated into a dataset from recent grassland and forest sites from Central and South-Eastern Europe (R. Zech et al., 2013). Degradation line for grass/herbs:  $y = 0.086 * \ln(OEP) + 0.4233$ . Degradation line for trees, with deciduous trees/shrubs:  $y = -0.123 * \ln(OEP) + 0.456$ .