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Plant wax n-alkane biomarkers in the tropical Andes (Ecuador)

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General discussion and synthesis

1 | RECAP

A detailed understanding of an ecosystem's past is necessary for effective governance and policymaking, especially in light of current land-use and climate change (Cuesta et al., 2009; Josse et al., 2009). It is therefore necessary to develop new tools, such as the *n*-alkane proxy, that can deepen our understanding of ecosystem history. Against the backdrop of the richness of the Ecuadorian Andes (Jørgensen and León-Yáñez, 1999), and the particular land-use and climatic change threats it's experiencing (Cuesta et al., 2009; Josse et al., 2009), I set out in this thesis to further our understanding of plant wax *n*-alkanes patterns and their application as a palaeoecological proxy. In particular, the overarching question addressed in this thesis is: **what do *n*-alkane patterns extracted from sedimentary records reflect?**

The major sources of variability in the *n*-alkane signal (i.e. what the *n*-alkane patterns reflect) can be classified into four P's: production, processes, preservation and protocol (Figure 2). Each of these P's were explored in separate data chapters: In the first data chapter (chapter 2) I focused on the *production* of *n*-alkanes within plants, in chapter 3 I focussed on taphonomic *processes*, in chapter 4 (having developed a deeper understanding of modern *n*-alkane patterns in the Andes in previous chapters) I explore what information was *preserved* in the ancient sedimentary *n*-alkane record at Erazo (easter Andean flank), and in the final data chapter (chapter 5) I explored *n*-alkane measurement *protocol* reproducibility. In order to answer the overarching question, three sub-questions were identified where the four P's interact:

- Do environmental or taxonomic factors dominate the *n*-alkane signal in a given context? (chapters 2, 3 and 4)
- Does the *n*-alkane signal degrade as the plant material degrades? (chapters 3 and 4)
- How much of the *n*-alkane variability can be explained by our protocols? (chapter 5)

I will now briefly review and discuss how my findings contribute to answering these sub-questions and conclude on answering the overarching question. In each section I will address limitations and list where there are opportunities for future research.

2 | DO ENVIRONMENTAL OR TAXONOMIC FACTORS DOMINATE THE *N*-ALKANE SIGNAL IN A GIVEN CONTEXT?

I find that the dominance of a taxonomic vs. an environmental signal in the *n*-alkane pattern shifts depends on the source material from which the *n*-alkanes were extracted. Consequently, I will break down the answer to this sub-question down into the four *n*-alkane sources studied: leaves, necromass, soils and sediments.

2.1 | Leaves

I find that there is a strong interplay between taxonomy and environment in the leaf n -alkane signal, that are not entirely distinguishable from each other. The results in chapter 2 show that the n -alkane patterns can vary in species-specific ways at the chain length level along the temperature and precipitation gradient. However, we also find that despite this variability, the relative abundance of longer ($>C_{29}$) n -alkanes increases with the environmental gradient. However, my results also suggest that n -alkane patterns differ between species independent of the environmental gradient, as two species sampled at the same site and of the same genus (*Miconia*) exhibited different n -alkane patterns. Taken together, my findings suggest that there is large variability in leaf n -alkane patterns, with a clear taxonomic component, but that a general environmental signal is reflected in the n -alkane patterns.

Similar results are found in other studies across ecosystems: despite the large variability in leaf n -alkane patterns at the taxonomic level, there is a consistent environmental signal in the (averaged) site leaf n -alkane datasets (Bush and McInerney, 2015; Carr et al., 2014; Feakins et al., 2016; Guo et al., 2014; Hoffmann et al., 2013; Sachse et al., 2006; Tipple and Pagani, 2013; Wang et al., 2018a, 2018b). Although early studies presented promising results chemotaxonomic value of leaf n -alkanes at the family level (Maffei, 1994, 1996b), Bush and McInerney (2013, 2015) suggested that in part, the large variability and overlap they observed across higher taxonomic levels and plant groups could be due to environmental conditions contributing variability to leaf n -alkane patterns of taxa. Another indication of the interplay between taxonomic and environmental factors, inserting large variability, in n -alkane patterns across plants was observed along an altitudinal gradient in Peru (Feakins et al., 2016). The n -alkane patterns of the sampled individuals varied extensively at each site, resulting in a very weak correlation between the change in n -alkane patterns (average chain length) and altitude. Only when the data was averaged at the site level, did the environmental signal in the leaf n -alkane pattern shifts become apparent (Feakins et al., 2016). Many studies confirm that the taxonomic and environmental signals in leaf n -alkane patterns are intertwined, which presents itself in the large variability observed in leaf wax n -alkane patterns across ecosystems.

Taken together the results indicate that the leaf n -alkane signal is dependent on what plants contribute to the n -alkane signal. Literature suggests that not all species are equally plastic in leaf wax n -alkanes properties (Sachse et al., 2006; Salatino et al., 1991; Tipple and Pagani, 2013). In particular, species that span across environmental gradients are likely to be more plastic in their leaf traits. Alternatively, genera (like *Miconia*) that are phylogenetically diverse are likely to introduce taxonomic variability in the n -alkane signal. The implication of the complex interplay between the environmental and taxonomic factors is that there is no single leaf n -alkane signal, and which dominates can only be accounted for if the input is known.

2.2 | Necromass

My results cannot conclude on which factor dominates the necromass *n*-alkane signal. The sampling along the environmental gradient was insufficient to say anything conclusive about an environmental signal. However, the data in chapter 3 does show that the *n*-alkane patterns varied to the same extent as leaf *n*-alkane patterns, indicating there could be overlap with the leaf signal.

The little available data on necromass *n*-alkane patterns suggest that the environment contributes to the variability of the necromass *n*-alkane signal, but the topic is understudied. Zech et al. (2011) presented evidence from leaf litter bag studies from three species (*Acer pseudoplatanus* L., *Fagus sylvatica* L. and *Sorbus aucuparia* L.) which show that the necromass *n*-alkane patterns of each species to vary with seasonality due to microbial activity. Additionally, Wu et al. (2019) find a trend that suggests that necromass patterns increase the contribution of longer chain *n*-alkanes (increase in average chain length) at lower elevation sites (i.e. warmer/wetter) in Peru. More work is needed in order to determine what factors determine the variability observed in the necromass *n*-alkane signal.

The available studies of *n*-alkanes in necromass tend to focus on recording changes in the concentration rather than recording changes in the composition of the *n*-alkane fraction (Wu et al., 2019; Zech et al., 2011). *n*-Alkane concentrations determine the input into soils and sedimentary records and therefore an important aspect of the *n*-alkane proxy. However, filling the knowledge gap in necromass *n*-alkane patterns is important to understand what the *n*-alkane patterns in soils and sediments reflect. In order to make any taxonomic or environmental inferences from soils and sedimentary *n*-alkanes based on *n*-alkanes extracted from leaves, it should hold that *n*-alkane patterns extracted from necromass are representative of their source material (i.e. living leaves). Potentially, expanding our knowledge on necromass *n*-alkane pattern variability could explain some of the inconsistencies observed in the soil *n*-alkane signal.

2.3 | Soils

The results in chapter 3 suggest that there is an environmental signal in the *n*-alkane patterns of soils. I find that soil *n*-alkane patterns increase the relative abundance of longer *n*-alkanes ($>C_{29}$) (as indicated by the average chain length, ACL) and decrease in the odd-over-even distribution of the *n*-alkane pattern (as indicated by the CPI) with temperature and precipitation. The multivariate analysis shows that the dominant shifts in the pattern occur in the odd-over-even predominance (CPI), suggesting that degradation of the signal is linked to the environmental conditions. Although my data was not designed to address any taxonomic factors, it shows a clear link between the soil *n*-alkane signal and environment.

Whether *n*-alkanes in soils reflect vegetation composition or environmental conditions is an active field of discussion, which in part is based around which metric is used

to characterize the *n*-alkane pattern change. To avoid the issue of metrics, one study that did not use summarizing metrics, but instead used the multivariate *n*-alkane and *n*-alcohol patterns to reconstruct upper forest line in the Ecuadorian Andes (Jansen et al., 2013, 2010). Multiple studies have proposed indicative ratios between soil *n*-alkane chain lengths (for example, the C_{31}/C_{29} ratio, the C_{29}/C_{27} ratio, the C_{33}/C_{29} ratio, and the $C_{31}/C_{29}/C_{27}$ ratio), mostly found to reflect to local vegetation structure (Bliedtner et al., 2018; Carr et al., 2014; Howard et al., 2018; Jansen et al., 2008; Kirkels et al., 2013). Additionally, the positive relationship between the soil ACL metric and temperature/aridity has been observed in other studies, which is often attributed to be indirectly related to the vegetation composition turnover (Carr et al., 2014; Guo et al., 2015; Tiplle and Pagani, 2013). Furthermore, other studies have observed an inverse relationship between temperature/precipitation and the soil CPI metric (Luo et al., 2012; Rao et al., 2009). It has been hypothesized that this is because microbial decomposition rates increase with temperature/precipitation, which is expressed in less odd-over-even distribution of soil *n*-alkane patterns (Luo et al., 2012; Rao et al., 2009). Taken together with my results, the picture emerges that what the *n*-alkane signal reflects, (partially) depends on which metric is used to describe the *n*-alkane patterns shifts.

Multivariate analysis (such as nMDS) proved to be very helpful with understanding what metric best described the dominant variability in the soil *n*-alkane patterns. Parsing the multivariate pattern prior to metricizing it, could potentially help explain why the results on soil *n*-alkane signal are mixed. It is possible that, depending on the metric, the *n*-alkane signal might inform vegetation shifts (ratios, ACL) rather than environmental conditions (CPI). However, future research is needed to understand how all kinds of soil processes affect the soil *n*-alkane signal (including spatiotemporal averaging, standing vegetation, environmental conditions and microbial alteration) and what metric best describes the shifts we observe in soil *n*-alkane patterns.

2.4 | Sediments

The *n*-alkane patterns from the Erazo section presented chapter 4 were interpreted as an environmental signal, independent of vegetation. I came to this interpretation based on: (i) the multivariate analysis showed that the Erazo *n*-alkane record was described by shifts in over-even-predominance (CPI), (ii) Wu et al identified the dominant *n*-alkane stocks along the Andean flank are in soils, (iii) the CPI described modern soils accurately, and correlated with the environment in chapter 3, (iv) CPI did not describe the leaf *n*-alkane patterns, so the metric is unlikely to be linked to vegetation composition, (v) pollen taxa indicative of cooling and reduced *n*-alkane degradation overlapped in the Erazo record, but were not linked. Taken together, I find this to be strong evidence for an environmental signal in sedimentary *n*-alkane patterns in the Erazo record.

Considering the mixed results in modern calibration data (sections 1.2.1, 1.2.2 & 1.2.3), it is perhaps unsurprising that the interpretation of sedimentary *n*-alkane patterns varies too.

All studies agree however, that some constraints on *n*-alkane input and complementary proxies are necessary to interpret the *n*-alkane pattern (Bush and McInerney, 2013; Carr et al., 2014; Hoffmann et al., 2013; Howard et al., 2018); which is a way of acknowledging the complexity and variability (including taxonomic and environmental) observed in modern leaf and soil calibration sets. Following the taxonomic evidence in modern calibration datasets (Maffei, 1996b; Mimura et al., 1998; Salatino et al., 1989), Jansen et al. (2010) interpret the *n*-alkane and *n*-alcohol records from a peat core to reflect fluctuations in vegetation composition. The reconstruction was based on the local standing vegetation biomarker (*n*-alkane and *n*-alcohol) patterns, which were fed to the VERHIB model. Although the calibration data did not take into account potential *n*-alkane variability due to species sensitivity to the environmental gradients (chapter 2), the taxonomic affinity was increased by taking into account the entire pattern (rather than particular metrics) and including both *n*-alkanes and *n*-alcohols in the model. Following the vegetation-environmental evidence (Carr et al., 2014; Guo et al., 2015; Tipple and Pagani, 2013), a record in Hawaii used *n*-alkane patterns to reconstruct vegetation shifts, which were interpreted to indirectly reflect shifts in environments (Crausbay et al., 2014). Following the soil CPI-environmental evidence (Luo et al., 2012; Rao et al., 2009) a study interpreted shifts in CPI during the late Pleistocene on the west Chinese Loess plateau as indicative of shifts in temperature/precipitation.

The picture painted by this literature is that the interpretation of the *n*-alkane patterns from sedimentary records depends highly on the sedimentary deposits studied (input source) and what part of the *n*-alkane pattern is interpreted (such as ratios, ACL and/or CPI). My work shows that it is possible to infer a meaningful environmental signal from sedimentary records. However, a deep understanding of *n*-alkane input and the dominant variability in the sedimentary *n*-alkane patterns was required to do so. Future studies hoping to use the *n*-alkane proxy should invest time and effort in grasping those aspects prior to attributing a taxonomic or environmental signal to the sedimentary *n*-alkane record.

3 | DOES THE *N*-ALKANE SIGNAL DEGRADE AS THE PLANT MATERIAL DEGRADES?

My results suggest that the *n*-alkane signal is altered, without losing the typical plant distribution, as the plant material degrades. In chapter 3 I find that necromass, soils and sediments *n*-alkanes reflect the typical higher terrestrial plant *n*-alkane distribution. I also find that the variability in soils is reduced, compared to leaf and necromass variability, suggesting some alteration of the signal. This was confirmed by the CPI metric, which multivariate analysis showed was an important descriptor of soil and sediment *n*-alkane patterns but not of leaf and necromass patterns along the transect (chapters 3 and 4). The CPI metric did not correlate with the ACL in soils, but did so in the Erazo sediments. The

increasing importance of CPI in describing the *n*-alkane patterns suggest that soil processes (degradation) play an increasingly important role in defining the *n*-alkane signal, and thus that the source material (plants) leave an decreasingly important imprint in the *n*-alkane signal.

My results fall in line with the available literature on *n*-alkane pattern taphonomy. Most studies agree that the *n*-alkane patterns in soils and sediments that show an odd-over-even predominance in the range of C15-C33 carbons reflects that of terrestrial higher plants (Eglinton and Hamilton, 1967; Jansen and Wiesenberg, 2017). However, two studies find microbial input in the mid-range *n*-alkane range (C₁₈-C₂₆) can contribute to the *n*-alkane patterns in necromass and soils (Brittingham et al., 2017; Zech et al., 2011), suggesting a distancing from the source the *n*-alkane signal. The positive correlation between CPI and ACL in sediments has been observed by other authors, and has led some studies to correct the ACL with the CPI (e.g. Buggle et al., 2010, and Zech et al., 2013).

Taken together, although it remains to be tested more explicitly, this indicates that there is clear increase in the importance of CPI as a descriptor of the *n*-alkane patterns, as the *n*-alkanes are sampled further away from their source material (i.e. leaves); where CPI is a poor descriptor of leaf wax *n*-alkanes (chapter 2, Bush and McInerney, 2013), it describes an important portion of the variability in soils (chapter 3, Howard et al., 2018), which eventually starts to affect the overall *n*-alkane pattern (i.e. the ACL) in sediments (chapter 4, Buggle et al., 2010). One way the contribution of degradation to the signal could be explored further is by quantifying the *n*-methyl ketones to *n*-alkanes ratio, as *n*-methyl ketones have been shown to be direct products of the *n*-alkane degradation process (Jansen and Nierop, 2009).

4 | HOW MUCH OF THE VARIABILITY CAN BE EXPLAINED BY OUR PROTOCOLS?

The analysis of the variability attributable to our protocols is understudied. Chapter 4 is a first attempt to characterize how much *n*-alkane measurements (i.e. homogenizing, extracting, fractioning, and measuring) can vary with the aim to initialize the discussion. The available data did not allow for a parsing of the variability, but it did showcase that *n*-alkane measurements can vary extensively. The question of how much variability in the *n*-alkane measurement is acceptable is highly dependent on the variability of the studied system. It also showed that *n*-alkane concentrations are measured less consistently than *n*-alkane patterns. However, it remains to be studied how this affects other *n*-alkane pattern characterizations (such as the ratios, ACL and CPI), as some metrics could be susceptible to small measurement inconsistencies due to their mathematical nature (an average could minimize small variations, whereas a ratio might exacerbate them). At its core, the work

presented in chapter 4 is a call to action to gather more data on *n*-alkane measurement reproducibility and to explicitly discuss how this affects our understanding of the *n*-alkane pattern proxy.

5 | WHAT DO *N*-ALKANE PATTERNS EXTRACTED FROM SEDIMENTARY RECORDS REFLECT?

The insights from (i) what the *n*-alkane signal reflects in any particular context, (ii) whether the *n*-alkane signal degrades at any stage, and (iii) how much of that variability can be explained by our protocols combined, have led me to the following conclusions:

- The overall *n*-alkane signal in soils and sediments is degraded, to the point that a chemotaxonomic signal (species, genus, family level) is unlikely to be traceable using *n*-alkanes alone. In particular when summarizing metrics like ACL are used in sedimentary *n*-alkane records. The contribution of microbes and other taphonomic processes is highly likely. This could be tested by checking for degradation signs (CPI, *n*-methyl ketones). The signs of degradation suggest that calibration datasets of leaf *n*-alkanes cannot directly inform what the sedimentary *n*-alkanes may reflect. Soil *n*-alkane records are likely better calibration datasets.
- The modern samples data from my thesis does not exclude the possibility for a vegetation structure (forest vs. grassland) signal in the sedimentary *n*-alkane record, although it was not observed in the Erazo section. In particular, I find that the work of Howard et al. (2018), Carr et al. (2014) and Jansen et al. (2008) are compelling examples of soil *n*-alkanes reflecting large-scale ecosystem structure. It remains to be studied if these findings in soils are also reflected in the *n*-alkane sedimentary record.
- The data in my thesis, and in the literature, shows that it is possible to identify an environmental signal in sedimentary *n*-alkanes. The CPI metric dominated the sedimentary record *n*-alkane pattern shifts, which have been hypothesized to reflect temperature/precipitation mediated by microbial degradation. However, this hypothesis is understudied as *n*-alkane taphonomy studies tend to focus on concentrations rather than *n*-alkane patterns. Additionally, a better understanding of the mechanistic link between environmental conditions, microbial activity, and CPI is needed.
- Understanding the *n*-alkane signal as a multivariate dataset is important, it underpins how the metrics will (accurately or not) characterize the *n*-alkane pattern shifts and signal.
- Understanding the variability introduced by our extraction and measurement protocols is essential, especially considering *n*-alkane patterns and concentrations exhibit large variability in natural ecosystems, across environments and plant taxa.

Taken together, there is much more that needs to be understood about the *n*-alkane signal, and the complex pathway it undergoes as it travels from leaf to sedimentary record. However, many studies are being done, showing promising results for the *n*-alkane pattern proxy. As our understanding grows, the *n*-alkane pattern proxy will help improve our reconstructions of past ecosystems and, ultimately, contribute to informing land use management practices, conservation policy and climate change.