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

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# 1

Introduction



# 1 | INTRODUCTION

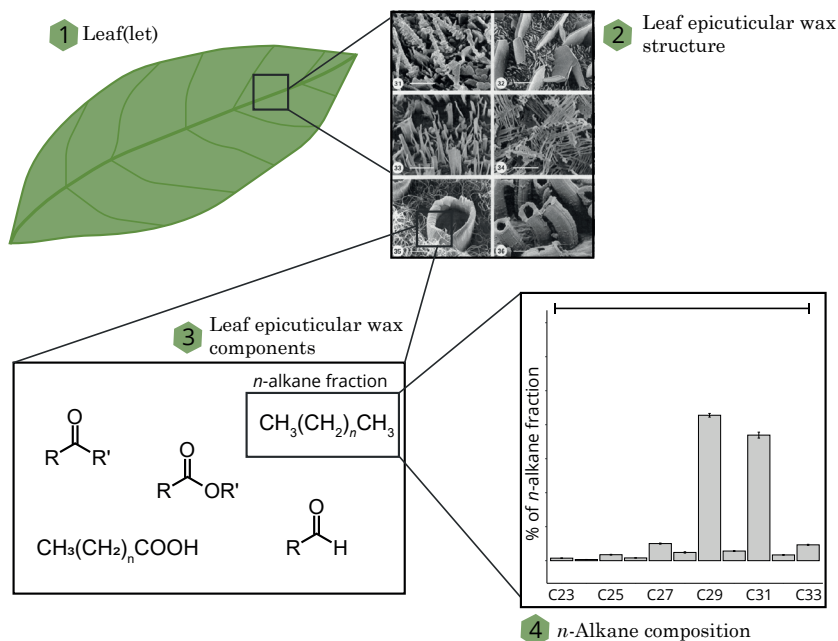
## 1.1 | Overarching rationale and research questions

Understanding how ecosystems have responded to changes in the past (such as the forest line moving downhill in cooler periods and vice versa (Villota et al., 2012) and the propagation of cultivar species and novel fire regimes with the arrival of humans (Maezumi et al., 2018)), can help predict how current ecosystems might change, and how habitable they might be, in the future. Therefore, to inform predictions, making accurate reconstructions of past ecosystems is important. More specifically an improved understanding of how ecosystems function, and vary, over the long-term (100-1000s years) can guide land-use management practices, conservation policy and climate change mitigation planning (Bush et al., 2014; Coffey et al., 2011; Willis and Birks, 2006). For example, the use of palaeoecological data to answer conservation questions on the Galápagos Islands related to native species and floristic composition (Bush et al., 2014; Coffey et al., 2011), and the use of palaeoecological data to inform forests restoration efforts in the Ecuadorian Andes (Jansen et al., 2013).

Ecosystems change over time and, by studying the remains they leave behind, it is possible to reconstruct what they looked like and how they changed on the long-term (Birks, 2019; Davis, 1981; Godwin, 1956; Smol et al., 2001). Sedimentary deposits, such as those found in lakes, accumulate ecosystem remains layer by layer through time. Examples of remains that can reflect ecosystem characteristics (also called proxies) are pollen, spores, charcoal, phytoliths, non-pollen palynomorphs, algae, and diatoms (Smol et al., 2001). For example, pollen analysis can be used to reconstruct variations in vegetation (Smol et al., 2001; von Post, 1916), particular non-pollen palynomorphs (coprophilous fungi) can provide evidence for mammal occurrence (Burney et al., 2003; van Geel, 2001), and charcoal analysis can be used to reconstruct variations in fire regimes (Bush et al., 2008; Whitlock and Larsen, 2001).

In order to further our understanding of past ecosystems and improve our reconstructions, new proxies are being developed. In particular, the utility of plant wax *n*-alkanes as a proxy for past vegetation cover has been explored over the last decades (Eglinton and Eglinton, 2008; Jansen et al., 2019; Jansen and Wiesenberg, 2017). *n*-Alkanes are remains of the leaf epicuticular wax from plants (Figure 1) and are found preserved in sedimentary deposits, peat sequences and palaeosols (Eglinton and Eglinton, 2008; Jansen et al., 2019; Jansen and Wiesenberg, 2017). Changes in the composition of *n*-alkanes (also known as the *n*-alkane pattern) can provide evidence for the input of higher terrestrial plants into sedimentary deposits (Eglinton and Hamilton, 1967) and to reconstruct past vegetation composition in soils and peat deposits (Jansen et al., 2006a, 2008, 2013, 2019).

In addition to controls on *n*-alkane composition relate to plant type, the *n*-alkane patterns of leaves, and soils, have also been found to fluctuate with environmental conditions

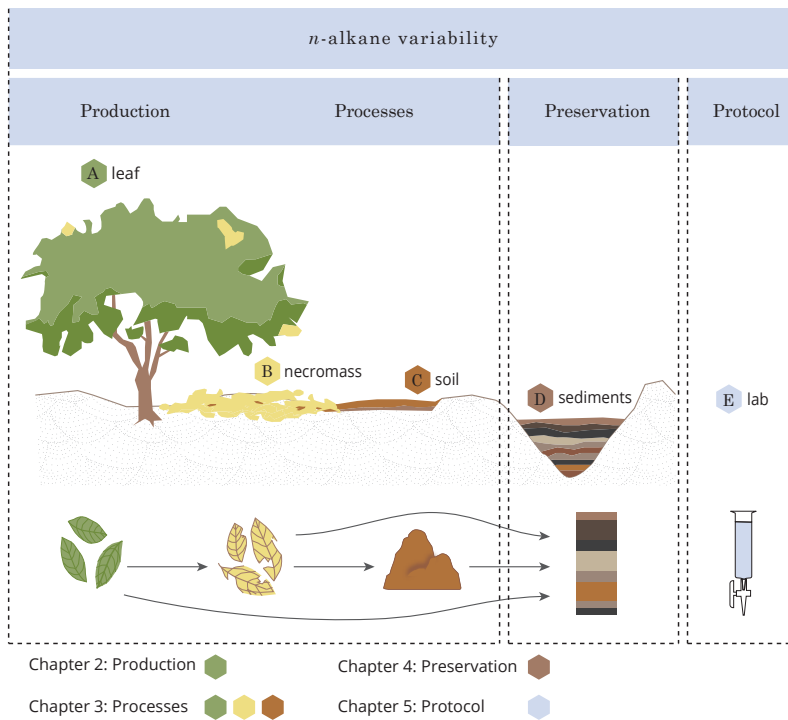


**Figure 1** | Leaf epicuticular wax *n*-alkanes. Diagram showing the leaf(let) (1), the leaf epicuticular wax (2), the most common leaf wax components (3) and an example of a typical leaf *n*-alkane pattern (odd-over-even distribution) (4). Image of leaf epicuticular wax structures from Eglington & Hamilton (1967).

(including temperature and moisture) (Bush and McInerney, 2015; Luo et al., 2012; Tipple and Pagani, 2013; Zech et al., 2011). Furthermore, other research suggests taphonomic processes and varying sedimentary inputs also contribute to the *n*-alkane patterns recovered from ancient material (Buggle et al., 2010; Ficken et al., 2000; Häggi et al., 2014; Jansen and Nierop, 2009; Nierop and Jansen, 2009). The multiple possible controls on *n*-alkane patterns evident from these new avenues of research pose the fundamental question: **what do *n*-alkane patterns extracted from sedimentary records reflect?** In order to answer this broad question, we need to (i) understand what factors contribute to the variability of *n*-alkanes in any particular context (Figure 2, **section 1.2.1**), and (ii) understand how the complex pathways that *n*-alkanes take, from their source (plants) to the sedimentary record, might influence what the *n*-alkane patterns extracted from ancient sediments can tell us about past ecosystems (Figure 3, **section 1.2.2**).

*n*-Alkane patterns extracted from leaves, necromass, soils and sediments vary in quantity and composition (i.e. how much is there? and what does it look like?, respectively), both within and between sample types (**section 1.2.1**). Many factors have been hypothesized to underly *n*-alkane variability (Diefendorf and Freimuth, 2017; Feakins et al., 2016; Jansen and Wiesenber, 2017), but they can be roughly classified under one of four P's: production, processes, preservation and protocol (Figure 2, **section 1.2.1**).

- *Production*: These factors control how much and which *n*-alkanes are being produced in plants.
- *Processes*: These are any factors that control the fate of *n*-alkanes in the (a)biotic environment.
- *Preservation*: These factors determine how *n*-alkanes travel into natural archives and how they are preserved once there.
- *Protocol*: These factors determine the lens through which we observe and capture *n*-alkane variability (our measurement capacities).

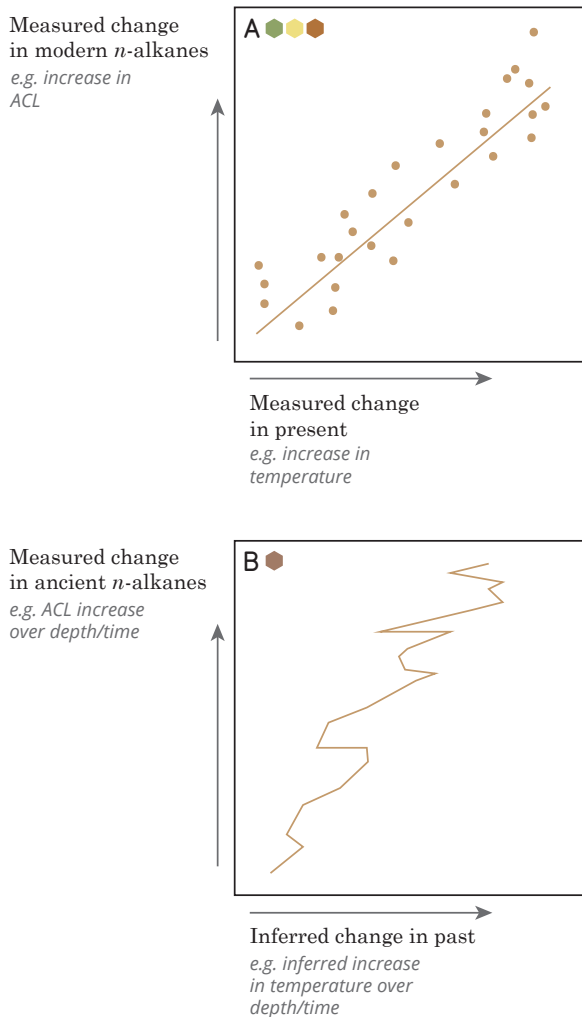


**Figure 2** | Conceptual framework of *n*-alkane variability used in this thesis. Factors contributing to *n*-alkane variability can be divided in production, processes, preservation and protocol. Production occurs in the plant (A green hexagons), processes of taphonomy and transport occur in leaf, necromass and soils (A green, B yellow, and C brown hexagons). Preservation occurs in sediments (D dark brown hexagons). Different lab protocols exist to extract and measure *n*-alkanes (E blue hexagons).

All these factors contribute to the observable *n*-alkane pattern variability in modern and sedimentary samples. However, to make inferences about past ecosystems based on *n*-alkane records, we need to identify what the *n*-alkane pattern shifts reflect (hereafter, the *n*-alkane signal) and which of the factors (if any) are the dominant driver behind the *n*-alkane pattern change (Figure 3, **section 1.2.2**)? For example, both the environmental conditions and the vegetation type are found to vary with the *n*-alkane patterns produced by plants

(for example, Carr et al., 2014; Hoffmann et al., 2013). Whether environmental or taxonomic factors accounts for most variability, and thus what the *n*-alkane signal is, remains an ongoing discussion (Jansen and Wiesenberg, 2017; Kirkels et al., 2013). We need to disentangle which factors are reflected in the *n*-alkane signal in order to interpret the ancient *n*-alkane records (Figure 3). Both our understanding of (i) the factors that control *n*-alkane variability (Figure 2), and (ii) what this variability reflects about ecosystems is limited (Figure 3). Key questions that remain unanswered are:

- Do environmental or taxonomic factors dominate the *n*-alkane signal in a given context?
- Does the *n*-alkane signal alter as the plant material degrades?
- How much of the *n*-alkane variability can be explained by our analytical protocols?



**Figure 3 |** Conceptual framework of *n*-alkane proxy interpretation. Leaf, necromass and/or soil *n*-alkane changes are linked to conditions in the present (A). This is used to interpret the measured changes in sedimentary *n*-alkanes (B). Hexagons indicate the *n*-alkane sources measured to infer these relationships, where green: leaf, yellow: necromass, brown: soil, dark brown: sediments.

### 1.1.1 | Study design

Mountains offer a great opportunity to further our understanding of the factors determining *n*-alkane variability in leaves, necromass, soils and sediments in the field. This is because mountain slopes induce environmental gradients in temperature, humidity, precipitation and other environmental factors (Becker et al., 2007; Malhi et al., 2010; von Humboldt and Bonpland, 1807). The tropical Andean region in particular provides a prime opportunity to study *n*-alkane variability because: (i) its high elevation means it contains long environmental gradients, and (ii) it has been subject to a relatively high number of previous *n*-alkane studies that can be built upon (Feakins et al., 2019; Jansen et al., 2013, 2010; Jansen and Nierop, 2009; Nierop and Jansen, 2009; Wu et al., 2019). In Ecuador, Jansen et al. (2013, 2010) developed the VERHIB model, based on extensive study of local vegetation, soil and peat *n*-alkanes and *n*-alcohols. In the Peruvian Andes, there are several studies examining *n*-alkane productivity and composition across plant communities, soils and sedimentary records (Feakins et al., 2018, 2016; Wu et al., 2017). Additionally, the *n*-alkane stocks in leaves, necromass and soils along the eastern Andean flank have been quantified (Wu et al., 2019). The environmental context and the availability of this foundational research offers a great opportunity to further our understanding of the *n*-alkane pattern proxy by expanding and embedding further work. For these reasons the tropical Andes is an ideal setting to study *n*-alkane patterns variability in leaves, necromass, soils and sediments.

The rationale for focusing on the tropical Andes is further strengthened when the pressing challenges the region faces related to human impact and on-going climate change are considered (Malhi et al., 2014) (**section 1.3**). The tropical Andes forms the largest mountain area in the tropics and has one of the world's highest levels of biodiversity and endemism (Josse et al., 2011; Myers et al., 2000). Furthermore, the ecosystems found on the tropical Andes harbour large amounts of carbon, therefore they play an important role in worldwide carbon sequestration (Calderón-Loor et al., in revision; Fehse et al., 2002). Both the biodiversity and carbon stocks in tropical regions are threatened by climate and land-use change (Cuesta et al., 2009; Josse et al., 2009; Nottingham et al., 2015). For example, increased grazing has increased carbon turn-over rates in high Andean ecosystems (Calderón-Loor et al., in revision). Understanding how these ecosystems have changed, what their resilience is, and how fast they can bounce back is vital to predicting how they will respond to current land-use and climate shifts.

In this thesis, I set out to shed light on the complex pathway that *n*-alkanes undergo from production in the plant to preservation in ancient sediments in the tropical Andes (Figure 2). The aim is to further our understanding of what *n*-alkanes extracted from sedimentary archives reflect about the past. Insights into this pathway will provide a better understanding of what *n*-alkane records can tell us about past ecosystems in general, and of the tropical Andean ecosystems in particular. In order to answer the overarching question, and specifically the three key questions posed in previous sections, my research

is presented in five main stages. First, I study the factors controlling the *n*-alkane signal in leaves from tropical tree species (production, chapter 2). Second, I study how the *n*-alkane signal transforms as it turns into necromass and into soils (processes, chapter 3). Third, I study the *n*-alkane signal from an ancient sedimentary archive (preservation, chapter 4). Fourth, I study how measurement error could be confounding our understanding of the *n*-alkane signal (protocols, chapter 5). Finally, I will use the knowledge obtained in the data chapters to answer the key questions and overarching question identified (chapter 6).

## 1.2 | Current understanding of *n*-alkanes

### 1.2.1 | What are plant wax *n*-alkanes?

Plant wax *n*-alkanes are part of the outer (epicuticular) wax layer found on plants, which forms a barrier between the plant and their environment. The epicuticular waxes can have different morphological structures, chemical compositions, and quantities (Figure 1). Different combinations of these properties can give rise to different functions, and thus the cuticular wax can provide several functional adaptations related to the environmental conditions in which the plant occurs (Koch and Ensikat, 2008). For example, one particular configuration of wax composition provides the self-cleaning properties of lily pads (Koch and Ensikat, 2008) while another wax configuration increases the hydrophobicity of *Brassica oleracea* leaves under dryer conditions (Koch et al., 2006).

Plant epicuticular waxes are a mixture of aliphatic hydrocarbons, mainly: alcohols, ketones, fatty acids, aldehydes and *n*-alkanes (Eglinton and Hamilton, 1967; Koch and Ensikat, 2008) (Figure 1). Although the *n*-alkanes comprise only a small fraction of the wax (compared to the other wax components), they are ubiquitous in plant cuticular waxes (Eglinton and Hamilton, 1967; Koch and Ensikat, 2008; Shepherd and Griffiths, 2006). The quantity and composition of the *n*-alkane fraction can vary. Although metricizing the quantity of the *n*-alkane fraction is straightforward (usually concentration by weight, for example: nanogram per gram of dry sample (ng/g of dry sample)), there are many ways to metricize the composition of the *n*-alkane fraction. The *n*-alkane fraction of the leaf wax is composed of multiple *n*-alkanes of different molecular chain lengths (i.e. more or less carbon atoms in the *n*-alkane), these typically range from 20 to 37 carbon atoms, and exhibit a strong odd-over-even predominance (Eglinton and Hamilton, 1967)(Figure 1). The particular configuration of these *n*-alkane chain lengths (i.e. the composition) is often also referred to as the *n*-alkane pattern or *n*-alkane fingerprint (Aebig et al., 2017; Eglinton and Eglinton, 2008; Jansen and Wiesenberg, 2017; Zech et al., 2009). The characteristics of the *n*-alkane pattern (and any changes therein) can be measured by multiple metrics, some common ones are: the average chain length (ACL) (Bush and McInerney, 2013), the carbon preference index (CPI) (Marzi et al., 1993), the odd-over-even predominance (OEP)(Bray and Evans, 1961; Scalan and Smith, 1970), and ratios between specific *n*-alkane chain lengths (for

example,  $C_{31}/C_{29}$ ,  $C_{33}/(C_{33}+C_{29})$  and  $C_{27}/C_{29}/C_{31}$  ratios) (Bush and McInerney, 2015; Carr et al., 2014; Jansen et al., 2008; Zech et al., 2013). Comparing metrics allows us to quantify overlap and differences in *n*-alkane patterns between individuals, species, vegetation groups, soils, and/or sediments. In addition, it is also possible to characterize the overall variability in the *n*-alkane patterns using multivariate (clustering) analysis (Maffei, 1994, 1996a; Tipple and Pagani, 2013; Vioque et al., 1996). All these different approaches to metricising and characterising the *n*-alkane pattern are necessary to provide a comprehensive insight into how *n*-alkane patterns vary, and determine what they potentially reflect about ecosystems.

### **1.2.1.1 | Production**

The *production* of *n*-alkanes is determined by many factors, roughly these can be divided in biotic and abiotic. The biotic factors are those thought to control *n*-alkane production at the plant level. For example, it is recorded that the leaf wax, and the *n*-alkane patterns, change during leaf ontogeny (Jetter and Schäffer, 2001). Similarly, the composition of the *n*-alkane fraction is known to change depending on what part of the plant is measured, e.g. roots vs. leaves, and along leaf venation (Gao et al., 2015; Jansen et al., 2006a). Different individuals can also have varying *n*-alkane quantities and composition (Bush and McInerney, 2013; Carr et al., 2014). Species can have different *n*-alkane quantities and composition production, although these differences are often not necessarily unique to a particular species, genus or family (Maffei, 1996b; Maffei et al., 2004; Mimura et al., 1998; Salatino et al., 1989). At a higher phylogenetic level, some general differences between *n*-alkane patterns and quantities of different plant groups have been observed, despite large overlap (Bush and McInerney, 2013). For example, *Sphagnum* mosses produce more short chain *n*-alkanes ( $C_{23}$  and  $C_{27}$ ) compared to the other plants (Bush and McInerney, 2013), and gymnosperms produce lower quantities of *n*-alkanes relative to angiosperms (Bush and McInerney, 2013; Diefendorf et al., 2011). The abiotic factors are those thought to determine *n*-alkane production externally. Considering the function of the plant cuticular wax, it is perhaps unsurprising that the *n*-alkane patterns have also been observed to vary with a myriad of environmental conditions, such as temperature, precipitation, humidity (Hoffmann et al., 2013; Huang et al., 2018; Maffei et al., 1993; Tipple and Pagani, 2013). For example, the leaf wax average chain length (ACL) in *Juniperus virginiana* and *Acer rubrum* were found to increase with temperature (Tipple and Pagani, 2013). Similarly, the *n*-alkane composition of *Rosmarinus officinalis* leaf *n*-alkanes has been found to vary with seasons (Maffei et al., 1993).

### **1.2.1.2 | Processes**

When plants stop producing *n*-alkanes, many types of degradation *processes* alter *n*-alkanes as they move from the plant into soils (taphonomic processes). Although *n*-alkanes are chemically stable compounds and they are thought to preserve well over time (Eglinton and

Eglinton, 2008; Jansen and Wiesenberg, 2017), they also contain valuable carbon resources that can be accessed by microorganisms. Studies find that the quantity and composition of the *n*-alkanes in necromass and soils alter over time (Sachse et al., 2006; Wiesenberg et al., 2004; Zech et al., 2011). This is hypothesized to be due to microbial activity, which are thought to consume the *n*-alkanes and contribute to the *n*-alkane fraction by producing more short (<C<sub>20</sub>) *n*-alkanes (Li et al., 2018; Zech et al., 2011), although lines of evidence suggest mid and long chain *n*-alkanes can also be produced by microorganisms (Ladygina et al., 2006; Li et al., 2018). Our understanding of the effect of microbial activity thus far suggests that microbes can alter the composition of the *n*-alkanes by preferentially consuming certain plant *n*-alkanes, or producing *n*-alkanes de novo without an odd-over-even predominance or a combination of both (Jansen and Wiesenberg, 2017; Luo et al., 2012). Additionally, there are multiple other factors that have been hypothesized to covary with *n*-alkane patterns measured in necromass and soils; such as temperature, moisture availability, pH, soil sorption and, (an)aerobic conditions (Li et al., 2018; Meyers and Ishiwatari, 1993; Quénée et al., 2004; Wu et al., 2019; Zech et al., 2011). For example, some studies have found that CPI values of *n*-alkanes extracted from soils decrease as site temperature increases, independent from standing vegetation (Luo et al., 2012; Rao et al., 2009). Although studies in necromass and agrarian soils suggest that *n*-alkanes have a turn-over of several months to decades (Wiesenberg et al., 2004; Zech et al., 2011), well preserved *n*-alkanes of plant origin are observed in natural archives extending over millennia and mega-annus (Bai et al., 2009; Peng et al., 2012; Wang et al., 2014). The debate about whether the overall *n*-alkane signal in soils and sediments reflects the environment and ecosystems within which they were originally produced, or whether they reflect post sedimentary processes, is on-going (Jansen and Wiesenberg, 2017).

### **1.2.1.3 | Preservation**

The factors determining how *n*-alkanes travel into the sedimentary record (input), and how they are *preserved*, is understudied. Extremely long term (c. 8 million years) preservation of plant derived *n*-alkanes in the sedimentary record has been demonstrated (Bai et al., 2009). However, whether (and to what extent) *n*-alkane patterns are altered, or reworked, after they are deposited in sedimentary archives remains unclear. One study shows negligible degradation of the *n*-alkane patterns in loess-palaeosol deposits dating from c. 71000 BP (Häggi et al., 2014). While other studies identify source dependent degradation of the *n*-alkanes in soils and peat deposits dating from c. 5600 and c. 4700 BP (Jansen and Nierop, 2009; Nierop and Jansen, 2009). The success of previous studies in extracting ancient *n*-alkane signals suggests that, over time, the *n*-alkane patterns should remain stable and reflect the environment from which they originate. However, this remains to be seen for other sedimentary settings especially as it seems that lipid, including *n*-alkanes, quantities

also covary with particle size and organic fraction of the sediments from which they are extracted (Aebig et al., 2017; Quénéa et al., 2004).

#### ***1.2.1.4 | Protocol***

The methods used to extract and measure the *n*-alkanes, the *protocol*, can also contribute to the variability we observe in the *n*-alkane signal (Aebig et al., 2017; Ardenghi et al., 2017; Jansen et al., 2006b; Quénéa et al., 2012). For example, a previous study found that the total lipid yield varied with solvent choice and temperature settings of the protocol (Quénéa et al., 2012). There is no standardized method for the way *n*-alkanes are sampled, processed, extracted and measured. Aside from differences in protocols, it is also possible that some protocols are more reliable than others – potentially adding to the variability observed in *n*-alkanes and hindering comparison between studies.

#### ***1.2.2 | What does the n-alkane signal reflect?***

The combined variability introduced by the *n*-alkane production, processes, preservation and protocol determine how the *n*-alkanes can be observed in the present, and ultimately, what information can be inferred from them about the past (Figure 2). The *n*-alkane signal, what the *n*-alkane pattern reflects about the ecosystem, can be different depending on what variable dominates in a particular context. This is why the interpretation of the ancient *n*-alkane signal is not straightforward, and explains why there are roughly three applications of the *n*-alkane pattern proxy: chemotaxonomic identification, reconstructions of vegetation structure (forest vs. grassland), and reconstructions of environmental (climatic) conditions.

#### **Chemotaxonomic signal**

One of the earliest applications of plant wax *n*-alkane patterns as a proxy was for the taxonomic identification of plants based on their chemical remains (chemotaxonomy) (Maffei, 1996b; Mimura et al., 1998; Sonibare et al., 2005). Many studies emerged characterizing the *n*-alkane composition of plant families (for example, Maffei, 1996a, 1996b, 1994; Salasoo, 1983; Salatino et al., 1989). The aim was to use the *n*-alkane patterns, in combination with other plant wax compounds, to identify plants that were no longer morphologically recognizable (Dove and Mayes, 2005, 1996; van Mourik et al., 2016). For example, in ruminant studies the *n*-alkane composition, together with other plant wax components, was used to identify the plant species consumed by sheep and goats after digestion (Dove and Mayes, 2005). Similarly, Jansen et al. (2013) developed a model that used *n*-alkanes and *n*-alcohols in combination with pollen to reconstruct the upper forest line of the northern Ecuadorian Andes during the late Holocene, which then informed sustainable reforestation efforts.

### **Vegetation structure signal**

*n*-Alkane patterns have also been used to reconstruct vegetation structures. In particular marine palaeoecologists identified *n*-alkanes as a proxy for terrestrial phytogeography, allowing to infer associated continental climatic conditions (Eglinton and Eglinton, 2008). The distinct plant distribution of the *n*-alkane patterns (odd-over-even), and the carbon-isotopic composition of the *n*-alkanes, were used to infer the photosynthetic pathway of the parent vegetation (Eglinton and Eglinton, 2008). Other studies used *n*-alkanes to reconstruct vegetation structure, as particular ratios between *n*-alkanes are thought to reflect forest vs. grassland input (Jansen et al., 2008; Zech et al., 2009).

### **Environmental (climatic) signal**

As modern calibration datasets emerged signalling that *n*-alkane patterns in plants reflected local environmental conditions (such as temperature and precipitation) (Bush and McInerney, 2015; Hoffmann et al., 2013; Tipple and Pagani, 2013) research focus shifted toward reconstructions of environmental conditions. For example, Crausbay et al. (2014) used *n*-alkanes to reconstruct drought and trade wind inversion in a 7300-year-old record in Hawaii, which, together with pollen and charcoal records, revealed dynamic vegetation driven by drought, fire and diebacks. Another study used *n*-alkanes, in combination with pollen records, to reconstruct relative changes in temperature and precipitation during the late glacial and Holocene periods in southern China (Zhou et al., 2005).

The differences in the application between chemotaxonomic, vegetation structure and environmental studies can be partially explained by the context of the study, for example the *n*-alkane input in the ruminant study is very limited (plant material of palatable species only) (Dove and Mayes, 2005), whereas in marine studies the input is predominantly aeolian or river sediments (Eglinton and Eglinton, 2008). However, the varying lines of evidence for taxonomic, vegetation or environmental signals in the *n*-alkane pattern in modern calibration studies also play a large part in the varying applications of the *n*-alkane proxy. This is because, despite efforts, the complex pathway of the plant *n*-alkanes prior to entering the sedimentary records, and thus what they reflect, is understudied (Figure 2, Figure 3). For example, there is an active discussion about whether the *n*-alkane patterns from sedimentary records reflect vegetation composition or environmental conditions, or which of the two dominates (Bush and McInerney, 2013). Additionally, efforts have intensified into studying the *n*-alkane patterns across plant taxa (Bush and McInerney, 2015, 2013; Carr et al., 2014; Diefendorf et al., 2015; Feakins et al., 2016; Hoffmann et al., 2013; Maffei, 1996a), but *n*-alkane pattern datasets from necromass and soils are comparatively less extensive (Häggi et al., 2016; Howard et al., 2018; Nierop and Jansen, 2009; Wu et al., 2019; Zech et al., 2011). Additionally, the extent to which the measurement error (i.e. the processing, extraction and measurement of the sample) influences our understanding of calibration

datasets or sedimentary *n*-alkane records, potentially leading to biased inferences, remains unknown.

### 1.3 | The tropical Andes

Being one of the largest continuous tropical mountain areas, the tropical Andes has some unique characteristics that make it an ideal place to gather calibration data on *n*-alkane variability along an environmental gradient. The tropical Andes range from c. 600 m to c. 6,000 m above sea level (m a.s.l.) (Josse et al., 2009), giving rise to characteristic environmental gradients in temperature (mean annual temperature range from -9 to 30 °C), and precipitation (annual precipitation range from 0.1 to 8,000 mm) (Fick and Hijmans, 2017; Josse et al., 2011). The environmental gradients, in turn, give rise to the five biomes in the tropical Andes: (i) the páramos, (ii) punas, (iii) montane forests, (iv) dry inter-Andean valleys and, (v) salt flats (Cuesta et al., 2009; Josse et al., 2009; Rivas-Martínez et al., 1999, 2011). Within these five biomes an estimated 133 distinct ecosystems have been identified; of which the montane forests cover much of the tropical Andes (at an estimated 24.6 % cover it is the largest ecosystem category of the North and Central Andes) (Cuesta et al., 2009; Josse et al., 2011, 2009).

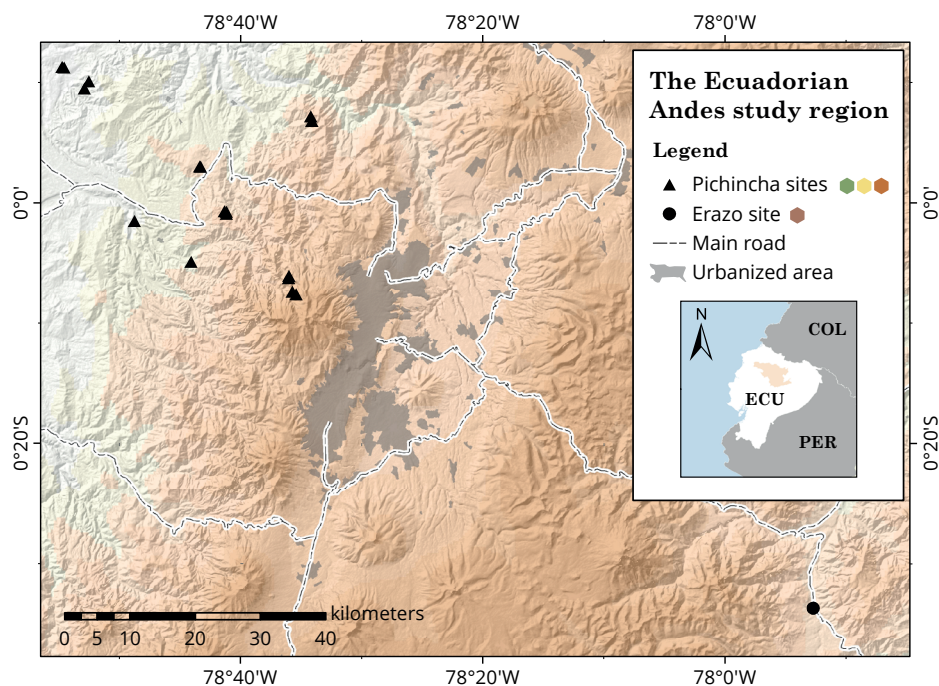
The montane forests of the tropical Andes fulfil several important roles, namely: providing water security and protection to more than 40 million people, controlling regional and continental climate, and holding between 20-40 tons of carbon per hectare making it an important sink of atmospheric CO<sub>2</sub> (Cuesta et al., 2009; Josse et al., 2009). These ecosystems, and their functioning, are vulnerable to changes in global climate and local land-use practices. For example, recent research found that 25% percent of the species on the montane forests of the tropical Andes are classified as highly vulnerable to warming, and another 25% are classified as moderately vulnerable (Cuesta et al., 2019). Thus, in order to effectively attempt to maintain healthy ecosystem functioning in the future, it is essential to fully understand the tropical montane forest ecosystems, and their resilience to climate change in particular.

Despite the need for a deeper understanding of the tropical Andes, the body of (palaeo-ecological) research done is relatively small. I chose to do the study in the tropical Andes, in part, because of the knowledge gap in this region. Additionally, the tropical Andes offer a steep environmental gradient over a short distance, making it an ideal testing ground for studying patterns in *n*-alkanes.

#### 1.3.1 | Study sites

I chose the Pichincha transect and the Erazo site for this thesis. The Pichincha transect is an established vegetation and carbon monitoring transect in the tropical Andes in Ecuador (Figure 4). The Erazo site is the source of Middle Pleistocene sediments (c. 190 thousand years old; Cosanga, Ecuador); the nearest known source of such ancient sedimentary mate-

rial to the Pichincha transect (Figure 4). The Pichincha and Erazo sites have similar vegetation composition today, and in the past, (lower montane, cloud forest, upper montane forest) and consequently provide the opportunity to explore modern and ancient *n*-alkane patterns.



**Figure 4** | The Ecuadorian Andes study region. The Pichincha transect sites are indicated by triangles, where the leaves, necromass and soils were sampled (green, yellow and brown hexagons). The Erazo site is indicated by a circle, where the Erazo sediments were sampled (dark brown hexagon). Beige shading on map indicates elevation, where darker colors indicate higher elevation (0 – 5920 m above sea level). Dashed lines with a white background indicate main roads. Grey shading indicates urbanized area (Quito).

### 1.3.1.1 | The Pichincha transect

I studied the *n*-alkane patterns along the tropical Andes by studying the Pichincha transect, located on the north western Andean flank of Ecuador (named after the Pichincha province it is located in) (Figure 4). The Pichincha transect was established for long-term monitoring of forest succession and carbon storage in the region, in 2015 by the research non-profit organization Consorcio para el Desarrollo Sostenible de la Ecorregión Andina (CONDESAN) (<http://condesan-ecoandes.org/>). CONDESAN collaborators catalogued many traits of the site and the trees on them. Among others, they identified and recorded each tree above 10 cm diameter at breast height, measured and monitored their size every year, catalogued key site features (like altitude, slope, soil properties and temperature and humidity), and

documented tree community composition at each site along the transect (Pinto et al., 2018). The transect captures multiple montane forest types (lower montane forest, cloud forest and upper montane forests) and environmental gradients in mean annual temperature (7.2- 21.6 °C), relative air humidity (96.1 - 99.8%) and mean annual precipitation (1580 - 2448 mm) (Karger et al., 2017). The Pichincha transect thus allows to study the production and processes of *n*-alkane patterns across a temperature, humidity and precipitation gradient.

### **1.3.1.2 | The Erazo site**

I studied the sedimentary *n*-alkane patterns and signal of the Erazo site sedimentary sequence. The Erazo site sequence was the focus a previous palaeoecological study by Cárdenas et al. (2014, 2011a, 2011b), who looked at the pollen, charcoal and macrofossil records from the sequence. Located just outside of Cosanga (Napo province, Ecuador) (Figure 4), at 1914 m a.s.l., the site is currently enveloped in lower montane forest vegetation. River and road cuttings reveal the sedimentary sequence, which was dated to the Middle Pleistocene (c. 324,000 - 193,000 years ago) (Cárdenas et al., 2011a). The sedimentary sequence is thought to have been deposited following a volcanic eruption that resulted in the valley becoming blocked, and allowed the accumulation of inter-bedded volcanic and organic deposits behind the volcanic 'dam'. Once the river cut through the dam, deposition stopped and subsequent down-cutting erosion exposed the sedimentary sequence (Cárdenas et al., 2014). Using the pollen, charcoal and plant macro fossils records, Cárdenas et al. (2014, 2011a) inferred the vegetation dynamics during the middle Pleistocene. In particular, the top section was interpreted as indicating that the vegetation drastically changed due to a cooling of the climate (Cárdenas et al., 2011a, 2014). The top section was resampled at high resolution in 2015, which opened up the possibility to describe the vegetation dynamics in more detail and complement the pollen records with the *n*-alkane pattern proxy. The extensive work done previously on the Erazo sites therefore offers a great context to study the application of the *n*-alkane pattern proxy in the Ecuadorian Andes.

## **1.4 | Thesis aim, scope and outline**

In this thesis I set out to shed light on what the *n*-alkane patterns extracted from sedimentary records reflect. More specifically, the three main research questions (RQ) addressed in this thesis are:

- Do environmental or taxonomic factors dominate the *n*-alkane signal in a given context? (RQ.1)
- Does the *n*-alkane signal alter as the plant material degrades? (RQ.2)
- How much of the *n*-alkane variability can be explained by analytical protocols? (RQ.3)

In order to answer these questions I focus on the factors and processes that have been observed to be important in the *n*-alkanes, as they transfer from leaf to the sedimentary record (Figure 2). Broken down per chapter, I address the research questions as follows:

- In chapter two I explore some of the factors controlling the production of *n*-alkane patterns from leaves (Figure 2A). Namely, I study whether different species respond similarly to three major environmental controls: temperature, relative air humidity, and precipitation. The hypothesis is that the composition of leaf wax *n*-alkanes of the species is plastic in species-specific ways. To test this I will generate a dataset of *n*-alkane patterns along the Pichincha transect, from six tropical tree species. On these data I will test whether there is a correlation between the *n*-alkane patterns (as indicated by the average chain length (ACL), the  $C_{31}/(C_{29}+C_{31})$  ratio and the individual chain lengths) of the species and the environmental gradient and whether these correlations have the same directionality. This chapter sheds light the taxonomic and environmental controls of the *n*-alkane signal in leaves (RQ.1).
- In chapter three I compare the *n*-alkanes extracted from leaves, necromass and soils to assess processes affecting the *n*-alkane patterns (Figure 2A,B&C). I particularly focus on whether the *n*-alkanes patterns are similar, and whether they reflect the environmental gradient they were sampled in. The hypothesis is that the *n*-alkane composition is similar and that the *n*-alkane signal of leaves, necromass and soils reflect the environmental gradient a similar way. I will test this by generating a dataset of *n*-alkane patterns from plants, necromass and soils sampled along the Pichincha transect. On these data I will test whether the *n*-alkane patterns overlap (using multivariate analysis), and whether there is a correlation between the environmental gradient and the *n*-alkane patterns of each sample type (as described by the ACL,  $C_{31}/(C_{29}+C_{31})$  ratio and CPI). This chapter furthers our understanding of the alteration of the *n*-alkane signal as the material degrades, and what the *n*-alkane signal reflects (RQ.1 & RQ.2).
- In chapter four I explore the application of the *n*-alkane proxy in a sedimentary record, in particular examining the input, preservation and interpretation of the *n*-alkane record (Figure 2D). It is anticipated that the *n*-alkane patterns are preserved in the sediments and reflect environmental conditions at the time of deposition. To explore this I will generate a dataset of the *n*-alkane patterns from the Erazo sedimentary record and a complementary pollen concentrations record. Multivariate analysis (unconstrained and constrained clustering), ACL, CPI and the proportion of aquatic input ratio (P<sub>aq</sub>) will expose dominant changes in the *n*-alkane along the record which will be interpreted using the results from previous data chapters and the previous work done on the Erazo sequence. This chapter contributes to our understanding of what the *n*-alkane signal reflects in sedimentary deposits (RQ.1 & RQ.2).
- In chapter five I shed light on an alternative source of *n*-alkane variability, namely the variability introduced due to the extraction and measurement protocols (Figure 2E).

Specifically, I look at the reproducibility of the protocol by quantifying the variation of replicate sample measurements (i.e. the repeated extractions and measurements of a homogenized sample). Due to the limited data available I focus on representing the variation in the replicate samples data, rather than statistical testing. However, I will test whether the *n*-alkane replicate data meet my definition of “acceptable reproducibility”: I will test whether the coefficient of variation of each replicate sample falls below the coefficient of variation of the studied system. I will also explore what the implications are of low protocol reproducibility on the findings and discuss potential sources of protocol variability. This chapter presents the effects of analytical protocols on *n*-alkane concentration and composition (RQ.3).

In the final chapter of the thesis I will answer each of the research questions separately, based on the current understanding and the results of the thesis. I will end the thesis discussing limitations and suggest further research, as indicated by the findings novel findings of this thesis.