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Species assembly on glacier forelands: Drivers of inter- and intra-specific trait variability.

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Table of Content

1. Einleitung (Deutsch)

Die Synökologie als Wissenschaftsrichtung befasst sich unter anderem mit Art-Abundanz-Mustern, Interaktionen zwischen Arten und Lebensgemeinschaften sowie dessen zugrundeliegenden Mechanismen. Da ökologische Prozesse auf verschiedenen räumlichen Skalen ablaufen, ist es wichtig zu verstehen wie diese miteinander verbunden sind, beziehungsweise nach welchen ökologischen Gesetzen Arten auf verschiedenen Skalen-Niveaus interagieren. Eine zentrale Fragestellung in dieser Hinsicht ist, zu verstehen welche Mechanismen hinter der Selektion einzelner Arten aus regionalen Metapopulationen liegen und wie sich daraus lokale Biozönosen bilden. Dieses Wissen bildet außerdem ein zentrales Element für den Naturschutz und die Modellierung der Auswirkungen des Klimawandels auf die biologische Vielfalt.

Im Zuge der Auseinandersetzung mit dem Thema entstanden viele Theorien, welche in drei Gruppen unterteilt werden können. Erstens, nehmen Neutrale Theorien an, dass alle Arten ein gleiches Maß an Fitness aufweisen und demnach durch eine idente Geburten- und Todesrate sowie Konkurrenzfähigkeit auszeichnet sind. Lokale Artzusammensetzung sind laut neutralen Theorien durch distanzabhängige Verbreitungs-, Besiedlungs- und Speziationsprozesse bestimmt. Abundanzen der einzelnen Arten entlang einer Zeitachse werden dabei besonders durch stochastische Prozesse und Störungsereignissen determiniert (Hubbell 2001, Bell 2001). Neutrale Theorien sind besonders für Ökosysteme mit starken Störungseinflüssen relevant und können als konzeptionelle Nullmodelle betrachtet werden. Zweitens, Nischen-basierte Theorien gehen besonders auf die Heterogenität der Umwelt ein und besagen, dass Arten durch unterschiedliche Eigenschaften bzw. Anpassungen an die Umwelt ausgezeichnet sind und demnach unter bestimmten Habitatbedingungen die jeweils höchste Fitness erreichen. Lokale Artengemeinschaften werden durch die Passung an lokale Habitatbedingungen aus dem regionalen Pool an Arten selektiert (Emerson & Gillespie, 2008). Diese Theorien gelten insbesondere für Habitate mit extremen Umweltbedingungen, dort findet ein besonders starker Selektionsdruck durch abiotische Faktoren statt. Die wegweisenden Arbeiten von Diamond (1975) bilden die Basis für die dritte Richtung an Theorien. Diese befassen sich insbesondere mit der Rolle von biotischen Interaktionen in Bezug auf die Selektion lokaler Artengemeinschaften und nennen hier Konkurrenz als determinierenden Faktor. Die relative Rolle dieser drei Faktorgruppen ist umstritten und variiert höchstwahrscheinlich je nach Biom und Ökosystem (Weiher et al., 2011).

Lange Zeit standen taxonomische Ansätze im Vordergrund der Synökologie, dabei wurde zum Beispiel nach nicht-zufällig auftretenden Artenpaaren in Gesellschafts-Matrizen gesucht, um Annahmen verschiedener Theorien zu testen (Gotelli & McCabe, 2002). In den letzten Jahrzehnten haben jedoch merkmalsbasierte Analysen als Methode in der Ökologie an Aufmerksamkeit gewonnen (Shipley, 2007). Während taxonomisch basierte Ansätze stark durch

historische Besiedelungsereignisse und Gegebenheiten beeinflusst sind, gilt dies weitaus weniger für merkmalsbasierten Analysen (Fukami et al., 2005; Temperton et al., 2013; Walker & Del Moral, 2003). Somit haben merkmalsbasierte Forschungsdesigns ein besonderes Potential verallgemeinerbare ökologische Regeln zu formulieren und die zugrundeliegenden Prozesse zu untersuchen. Betrachtet man nun Ökosysteme über mehrere Skalenniveaus und breite ökologische Gradienten hinweg, so können durch integrative Ansätze die Vorteile beider Herangehensweisen genutzt werden (Fukami et al., 2005). Das Forschungsfeld der merkmalsbasierten Ökologie weist ein großes Potential auf, steht jedoch auch vor kritischen Schlüsselfragen. So erweisen sich die Selektion und die Messung möglichst aussagekräftiger Merkmale oft als große Herausforderung. Wichtige Kriterien sind, dass Merkmale entlang ökologischer Gradienten deterministisch variieren und in direktem oder indirektem Zusammenhang mit der Fitness von Arten stehen. Besonders in Hinsicht auf Fragestellungen bezüglich des Einflusses ökologischer Filter und artspezifischer Einnischung auf die Zusammensetzung von Biozönosen, ist die richtige Wahl an Merkmalen besonders entscheidend. Da robuste und verallgemeinerbare Beziehungen zwischen Merkmalen und Umweltgradienten nicht immer leicht zu erkennen beziehungsweise zu messen sind, stellt dies die Wissenschaft vor große Herausforderungen (Funk et al., 2017; Shipley et al., 2016). Während entlang globaler ökologischer Gradienten (Díaz et al., 2016) beziehungsweise den wichtigsten öko-evolutionären Entwicklungen (Zanne et al., 2014) Merkmal-Umwelt-Beziehungen offensichtlich sind und biogeografische Auswirkungen haben (Stahl et al., 2014), findet man auf kleineren räumlichen Skalen oft nur idiosynkratische Zusammenhänge (Albert et al., 2010). Dieser Tatsache geschuldet hat die Rolle der räumlichen Skala in diesem Forschungsfeld stärkere Aufmerksamkeit erfahren und bildet somit neben der korrekten Wahl von Merkmalen, der Erfassung gesamter ökologischer Gradienten und inter- als auch intraspezifischer Merkmalsausprägungen, eine weitere Dimension der Komplexität (Blonder et al., 2018; Chalmandrier et al., 2017; Messier et al., 2010; Stark et al., 2017).

Seit dem Ende der Kleinen Eiszeit (LIA) im Jahr 1859 unterliegen die Gletscher der Zentralalpen einem kontinuierlichen Rückgang (Fischer, 2016; Lieb & Kellerer-Pirklbauer, 2018, 2019, 2020, 2021). Neben dem Volums-Rückgang, verlieren die Gletscher auch an Länge, was zur Ausdehnung der Gletschervorfelder führt. Die Rohböden des einstmals vergletscherten Terrains sind frei von jeglichen Samenpools und unterliegen primären Sukzessionsprozessen. Durch die raum-zeitlich gestaffelte Anordnung sind Gletschervorfelder ideale Lebensräume, um die Entwicklung von Lebensgemeinschaften zu untersuchen. Zusätzlich unterscheiden sich benachbarte Lebensgemeinschaften entlang der Chronosequenz erheblich im Artenreichtum, der Artenzusammensetzung und der Vegetationsdeckung (Raffl et al., 2006; Raffl & Erschbamer, 2004).

In dieser Studie befasse ich mich mit den Faktoren, die für die spezifische Zusammensetzung von Pflanzengemeinschaften verantwortlich sind und nutze dafür die besonderen Beschaffenheiten von Gletschervorfeldern. Im Fokus stehen hier die beiden Blatt-Merkmale Spezifischer Blattflächenindex (SLA) und Blattstickstoffgehalt (LNC) der drei Zielarten *Geum reptans, Oxyria digyna* und *Persicaria vivipara*. Entlang von drei Gletschervorfeldern der Zentralalpen wurden auf 85 Aufnahmeflächen á 1m² Blatt-Merkmale gemessen und Vegetationsaufnahmen nach Braun-Blanquet (Braun-Blanquet, 1964) erhoben. Die Aufnahmeflächen sind in Transekten transversal zum Verlauf der Chronosequenzen angeordnet, um so die Entwicklung der Pflanzengemeinschaften zu erfassen. Die beiden ausgewählten Blatt-Merkmale stellen Indikatoren der ökologischen Strategien von Arten und Individuen bezüglich Ressourcen - Akquise und – Speicherung dar. Das Merkmal SLA lässt dabei auf einen Trade-Off zwischen maximierter Stoffwechselaktivität (hohe Werte) und Stress-Toleranz (niedrige Werte) schließen. LNC hingegen bezieht sich auf die Balance zwischen hoher photosynthetischer Kapazität und die dafür notwendige aktive Stickstoff-Akquise (z.B. durch Mykorrhiza oder Priming) beziehungsweise Stickstoff-Aufnahme. Betrachtet man die drei Zielarten, so sind *Geum reptans* und *Oxyria digyna* typische Pionierarten von Gletschervorfeldern, *Persicaria vivipara* hingegen ist eher als Generalist zu betrachten (Adler et al., 1994). Keine der drei Zielarten erreicht jedoch in späten Sukzessionsstadien und Habitaten mit geschlossener Grasnarbe hohe Abundanzen. Alle drei Arten können daher als schwache Konkurrenten betrachtet werden.

Meine erste Forschungsfrage befasst sich mit dem Thema, welche Faktoren dazu führen, dass Blattmerkmale über verschiedene Skalenniveaus hinweg variieren. Dabei wird in der vorliegenden Arbeit zunächst die relative Verteilung zwischen inter- und intraspezifischer-Variabilität der beiden Merkmale betrachtet. Zusätzlich wird die Partitionierung der Varianz über die ökologischen Skalen-Niveaus des Versuchsdesigns hinweg untersucht, um Hinweise auf starke Umwelt-Filter zu prüfen. Da auf Gletschervorfeldern extreme Lebensbedingungen herrschen, ist zu erwarten, dass Umwelt-Filter als primär treibende Prozesse auf die Bildung und Zusammensetzung der Zönosen wirken (Albert et al., 2010; Kichenin et al., 2013). Da die räumliche Skala der transversalen Transekte die Entwicklung der Gletschervorfelder widerspiegelt und sich entlang des Alterungsprozesses bzw. Höhengradientens die abiotischen Umweltbedingungen am stärksten ändern, erwartete ich, dass innerhalb der einzelnen Arten die meiste Varianz zwischen den Transekten und die geringste Varianz zwischen den Plots zu finden sein wird.

Zweitens beschäftige ich mich in dieser Arbeit mit der Frage, durch welche Umwelt-Parameter die Blatt-Merkmale SLA und LNC determiniert werden. Um dies zu beantworten, habe ich die gemessenen Blatt-Merkmale mittels linearer Modelle mit Indikatoren für biotische und abiotische Parameter der Aufnahmeflächen korreliert. Da eine umfangreiche Auswahl an Indikatoren für diese Berechnungen gewählt wurde, erwarte ich konkrete Zusammenhänge zwischen Blatt-

Merkmalen und Umweltfaktoren zu finden. Insbesondere erwarte ich, dass die Ausprägung der Merkmale entweder durch ein Set an abiotischen Parametern, assoziiert mit Nischen-basierter Selektion, oder durch Konkurrenz bzw. Parametern biotischer Interaktion erklärt werden. Aufgrund der Strukturierung der Gletschervorfelder ist eine starke Wirkung abiotischer Stressoren besonders in den frühen Sukzessionsstadien zu erwarten, während mit zunehmendem Alter der Sukzessionsfläche die Bedeutung von Interaktionen, insbesondere der Konkurrenz steigen sollte.

2. Manuskript:

Abstract

Understanding the factors which govern the assembly of species from regional metacommunities into local species assemblages is central to community ecology and important for predicting the impact of climate change on biodiversity. In this study, I investigate the mechanisms behind community dynamic assembly processes on three glacier forelands in the Austrian Alps. These successional sites provide chronosequences and a hence unique opportunity for a space-for-time substitutions approach. I asked how plant traits vary within and between nested levels of ecological organization and which filters play a dominant selective role in these ecosystems. Therefore, I investigated the variation and response of two traits (specific leaf area index (SLA) & leaf nitrogen content (LNC)) of three alpine plant species (*Geum reptans, Oxyria digyna* & *Persicaria vivipara*) along three glacial chronosequences of the Venediger Mountain Range. Results indicate a moderate link between the two traits and environmental conditions as well as high trait divergence at smaller ecological levels of organization. Possible explanatory models are the presence of favorable microhabitats within plots, a high relevance of stochastic effects during early stages of colonization, insufficient time for niche filtering processes or relevance of biotic interactions. Additionally, results show a non-negligible proportion of intra-specific variation in both traits, which is especially true for the chemical leaf trait LNC, where variation between individuals of the same species accounts for more than 50% of the total variation.

Keywords

glacier foreland, intra specific variability, leaf nitrogen concentration, micro habitats, plant traits, specific leaf area, species assembly

Abstract (Deutsch)

Ein wichtiges Forschungsfeld der Synökologie ist zu verstehen, welche Mechanismen zur Selektion lokaler Populationen aus regionalen Artenpools führen. Dies ist nicht nur von zentraler Bedeutung für die Erforschung von Lebensgemeinschaften, sondern auch für die Vorhersage der Auswirkung des Klimawandels auf die biologische Vielfalt. Meine Studie beschäftigt sich mit den Faktoren, welche die Zusammensetzung von Pflanzengemeinschaften alpiner Sukzessionsflächen determinieren. Konkret wird die Variation von zweit Blatt-Merkmalen (spezifischer Blattflächenindex (SLA) & Blattstickstoffgehalt (LNC)) dreier alpiner Pflanzenarten (*Geum reptans, Oxyria digyna* & *Persicaria vivipara*) entlang ökologischer Gradienten und Organisationseinheiten untersucht. Durch den Rückgang der Gletscher seit dem Ende der Kleinen Eiszeit entstanden Sukzessionsflächen, die einmalige Freilandlabore für synökologische Fragen darstellen. Die Resultate meiner Analysen zeigen einen mäßigen Zusammenhang zwischen den beiden Blatt-Merkmalen und den erhobenen Umweltbedingungen sowie ein hohes

Maß and Divergenz auf kleinen ökologischen Organisationsebenen. Für die hohe Variabilität zwischen und innerhalb der Aufnahmeflächen eines Transekts kann es mehrere Gründe geben. Mögliche Erklärungsmodelle sind das Vorhandensein von begünstigten Mikrohabitaten innerhalb der Relevés, eine hohe Relevanz stochastischer Effekte, unzureichend Zeit für Nischen-basierte Selektion oder hohe Relevanz von biotischen Interaktionen. Insbesondere durch die Ergebnisse des chemischen Blattmerkmals LNC, bei dem die intraspezifische Variabilität mehr als 50% der Gesamtvariation ausmacht, kann mit dieser Arbeit die Bedeutung der Merkmalsvariation innerhalb einer Art für synökologische Fragestellungen hervorgehoben werden.

Schlagwörter (Deutsch)

Artenzusammensetzung, Blattstickstoffkonzentration, Eigenschaften von Pflanzen, Gletschervorfelder, Intraspezifische Variabilität, Mikrohabitate, Spezifische Blattfläche (SLA)

Introduction

Understanding the factors which govern the assembly of species from regional metacommunities into local assemblages is central to community ecology and important for predicting the impact of climate change on biodiversity. Ecological theory distinguishes three main groups of processes that determine community assembly. First, neutral theory abstracts from species-specific differences and tries to understand local species composition as a product of distance-dependent dispersal and recruitment processes (Hubbell 2001, Bell 2001). Second, niche-based concepts emphasize the role of local environmental conditions as filters that select a subset of species with appropriate traits from the regional metacommunity (Emerson & Gillespie, 2008). And third, another thread of theory, starting with the seminal work of Diamond (1975), highlights the role of biotic interactions, especially competition, for determining local species composition. The relative roles of these three factors or factor groups are contentious and most probably vary across biomes and ecosystems (Weiher et al., 2011).

For a long time, researchers focused primarily on a taxonomic approach, e.g. by searching for complementary species pairs in community matrices (Gotelli & McCabe, 2002), to answer questions about species assembly processes and community composition. However, over the past decades, trait-based community ecology has gained increasing attention in this context (Shipley, 2007). Whereas taxonomically-based approaches can be strongly influenced by historical contingencies due to priority effects of colonization (Fukami et al., 2005; Temperton et al., 2013; Walker & Del Moral, 2003), trait-based approaches abstract from these contingencies and allow for analysing more generic ecological patterns. Nevertheless, for an integration across local and regional scales, both trait-based and taxonomic approaches can provide important contributions (Fukami et al., 2005).

To analyse the impact of species' niches on community assembly, trait-based ecology should focus on traits which deterministically vary along ecological gradients and can thus be interpreted as ecological filters. However, robust relationships between species traits and environmental gradients are not always easy to identify (Funk et al., 2017; Shipley et al., 2016). While relationships between traits or trait states and global ecological gradients (Díaz et al., 2016) or major eco-evolutionary developments (Zanne et al., 2014) are obvious and have important biogeographical implications (Stahl et al., 2014), more idiosyncratic trait-environment relationships are often found at smaller spatial scales (Albert et al., 2010). As a consequence, the role of spatial scale in trait-environment relationships has increasingly attracted attention (Blonder et al., 2018; Chalmandrier et al., 2017; Messier et al., 2010; Stark et al., 2017).

While many studies have focused on mean trait responses along environmental gradients, the extent of intraspecific trait variability (ITV) has long been assumed to play a rather negligible role in the context of trait-based ecology (Shipley et al., 2016). However, recent research has provided evidence that ITV can account for a significant proportion of the total variance of traits within communities, especially in species-poor communities and at smaller scales (Auger & Shipley, 2013; Chalmandrier et al., 2017; De Bello et al., 2021; Messier et al., 2010; Violle et al., 2012). Summarizing this work, a global meta-analysis of Siefert et al. (2015) found that ITV accounted for 25% of total trait variation on average and that within community ITV decreased with increasing species richness. These results are supported by studies that found that if ITV is neglected, estimates of community-weighed mean traits may be biased, and species' ecological niches underestimated (Albert et al., 2012; Carmona et al., 2015; De Bello et al., 2021; Jung et al., 2010; Siefert et al., 2015; Westerband et al., 2021).

The glaciers of the Central Alps have been subject to continuous deglaciation since the end of the Little Ice Age (LIA) in 1859. As a consequence, glacier forelands expand every year simultaneously initiating primary succession processes on the deglaciated terrain. The glacier foreland hence offers chronosequences which allow following community assembly process by means of space-for-time substitutions. The neighbouring communities vary considerably in species richness, species composition and total species cover (Raffl et al., 2006; Raffl & Erschbamer, 2004).

In my study, I used these properties of glacier foreland vegetation to ask several questions relevant to trait-based community ecology. I sampled two traits, specific leaf area (LAI) and leaf nitrogen content (LNC), of three species co-occurring in 1 m²-plots arranged as transects across the chronosequence of three different glacier forelands in the Austrian Alps. Variation in the two selected traits indicate different leaf strategies of resource capture and conservation. SLA mainly relates to a trade-off between rapid carbon gain, often associated with a competitive strategy, and a strategy that maximizes stress tolerance and longevity. Variation in LNC, by contrast, is related to the trade-off between high photosynthetic capacity and the costs associated with the acquisition

of nitrogen and increased attractiveness to herbivores. Two of the three species, *Geum reptans* and *Oxyria digyna*, are typical pioneers of glacier foreland vegetation and the third one, *Persicaria vivipara*, is a generalist of alpine vegetation (Adler et al., 1994). None of the species usually dominates in closed swards, and they can hence all be considered weak competitors.

My first question relates to how the variation in functional traits in and across these three species is distributed across nested levels of ecological organization from the glacier-foreland level down to the level of different individuals within a plot. As niche-filtering is commonly assumed to play a dominant role in community assembly in stressful and extreme environments like these forelands (Albert et al., 2010; Kichenin et al., 2013), I expected to find most variation attributable to the level of transects, and least to variation in supposedly homogenous plots – at least among individuals of the same species. My second question asks which features of individual 1 m² plots determine the mean trait states of individuals growing in these plots. I therefore combined features that indicate properties of the abiotic and the biotic environment. The rationale behind is that when biotic interactions or niche filtering is the dominant control of community assembly, these trait states should be more responsive to either one or the other group of indicators. In particular, I expected to see relationships of traits either with environmental indicators of abiotic stress and resource shortage in pioneer communities, associated with low SLA and LNC; or with indicators of increasingly competitive environments along the chronosequences from pioneer to grassland communities, associated with higher SLA and LNC.

Material and Methods

Study area

Data collection and plant surveys were conducted on three glacier forelands of the Venediger mountain range (Fig.1d) (Salzburg and Tyrol, Austria, 47° 6′ N, 12° 20′ E) during the vegetation period 2016 (June-August). The survey sites were located in the Viltragenvalley ("Viltragental", Fig.1a), in the Frosnitzvalley ("Frosnitztal", Fig.1b) and in the Untersulzbachvalley ("Untersulzbachtal", Fig.1c) and correspond to those of Schwienbacher et al. (2018). Geologically, most of the study area belongs to the Tauern window and consists mainly of acidous centralgneis. In the Frosnitztvalley base-rich strata emerge at the southern boundary of the glacier foreland. The three forelands are close to each other, but differ in some aspects of the environment. In particular, base elevations are not the same, and data were hence collected at elevations ranging from 1840 to 2950 m asl. Moreover, glacier retreat since 1859 differed in rates, but with fluctuations among the forelands. To give an example from the recent past, the average loss of glaciers in the Venediger mountain range was 40.5 m in 2018 and 24.4 m in 2020, while the Viltragenkees (glacier located in the Viltragenvalley) lost 128 m in length in 2018, and Untersulzbachkess (glacier located in the Untersulzbachvalley) 44 m (Lieb & Kellerer-Pirklbauer, 2019, 2021).

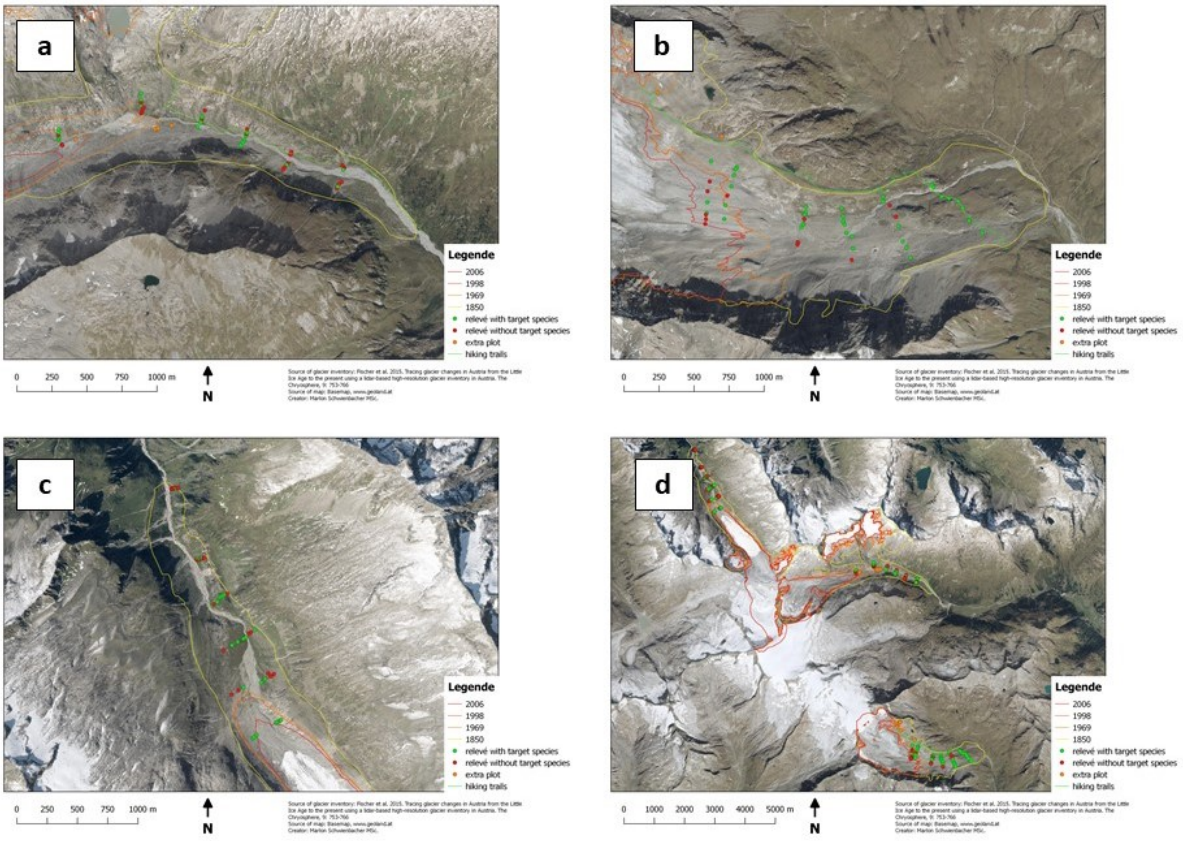

Figure 1a-d: This figure shows the three sampling sites (a: Viltragenvalley, b: Frosnitzvalley, c: Untersulzbachvalley) and an overview of the Venediger Mountain Range (d). The glacier extents of 1850 (yellow), 1969 (orange), 1998 (dark orange) and 2006 (red), as well as the hiking trails (green). The Relevé's are classified into three classes -relevés with target species (green), relevés without target species (red) and extra plots (orange) (adapted from Schwienbacher, 2018*).*

Sampling methods

I sampled communities in these three forelands such that all successional stages, ranging in age from 5 to 166 years (end of the Little Ice Age (LIA)), were represented. Thereby, six transversal transects, with eight 1m² plots each, were sampled on all of the three glacier forelands (Raffl et al., 2006; Raffl & Erschbamer, 2004). Each plot along the transect was randomly selected by throwing a ball blindly into the air, with the landing position then defining the upper left corner of the plot (upper side facing the glacier). When a target species (*Geum reptans*, *Persicaria vivipara*, *Oxyria digyna*) was present in a plot, up to three mature leaves of each species were sampled from up to three individuals and stored in an herbarium field press. In addition, all vascular plants in the plots were recorded, together with their cover-abundance values, according to the method of Braun-Blanquet (1964). To achieve an approximately logarithmic scale for the estimated plant cover values, the cover-value "2" (plant cover of 5-25%) was refined into "2a" (5-12.5%) and "2b" (12.5-25%) according to Dierschke (1994). The estimate-value "r" was used only when fewer than four individuals of a rare species were found in a plot (and if the species was very rare in the

surrounding area). Additionally, moss and lichen cover were noted if they exceeded the 5% threshold.
For repeatability purposes, the exact location and position of each relevé was recorded using a GPS-device and photography-documentation.

Specific leaf area

Leaf samples were stored and dried in a drying chamber at a constant temperature of 60°C for at least 14 days. For reasons of consistency and repeatability the rhachis of each leaf was cut at the edge of the lamina. This step of preparation reduces distortion of the leaf area due to positive phototropic length growth. Subsequently, the samples were scanned with a commercially available scanner and leaf areas were determined with the software imageJ (Schneider et al., 2012). Dry weight of all samples was measured using a precision balance. The specific leaf area (SLA) is the ratio of a leaf's area to its dry mass. All SLA values are given as m^2 kg-1.

Nitrogen concentration

Analysis of leaf nitrogen concentrations (LNC) was performed at the plant individual level. To obtain homogenized samples, all leaves sampled from a plant individual (1-3 leaves) were pooled and ground with a ball mill.
Between 1,5 and 2 mg of leaf material was weighed into tin capsules using a highly sensitive mass balance. Nitrogen concentrations of all prepared samples were then analysed with a Carlo Erba 1110 Elemental analyser coupled to a Finnigan Delta Plus Isotope ratio mass spectrometer (by Thermo Fischer) using a continuous gas flow interface (Conflo III).

Statistical analysis

Statistical analysis was done using R 4.0.3 (R Core Team, 2020), the nlme- (version 3.1, Pinheiro et al., 2020), the ape- (version 5.0, Paradis & Schliep, 2018), the piecewiseSEM- (version 2.0, Lefcheck, 2016), the ggplot2- (version 3.3.5, Wickham 2016), and the ggpubr-package (version 0.4.0, Kassambara 2020). The full reproducible code is available in Supplementary Material.

Variance components and relative importance of intraspecific trait variation

To analyse my first question on the partitioning of trait variation among levels, or scales, of ecological organisation, I followed a twofold approach based on the work of Messier et al. (2010). I fitted linear mixed-effects models (lme:nmle) of $\log_{10}$-transformed specific leaf area (SLA) and $\log_{10}$-transformed leaf nitrogen concentration (LNC) data to the variance (i) across and within species, and (ii) across the consecutive nested scales of single species data, and then calculated the variance partitioning between given scales. To technically implement the two different organizational structures, I used random factors of the linear mixed-effect models defined

according to the structure of my research question. To get insights into the variation of traits (i) between and within species, the random factor structure of the cross-species models was set to *random = ~1|species*. In addition to this approach, I analysed (ii) the relative importance of consecutive nested ecological scales for the expression of individual species traits, i.e. this approach represents a further partitioning of the intraspecific trait variation among ecological scale units. Therefore, I used the following nested random factor structure for the three single-species models *random = ~1|glacier/transect/plot*. In the case of SLA, I could have further partitioned the variation at the level of leaves within individuals. However, to be consistent across the two traits data, I did not consider this lowest level in the SLA analysis, i.e. I calculated the mean of all sampled leaves per individual and used it in the analysis. In a second step, I calculated the variance components of the observed traits for both approaches, given as the percentage of the total variance associated with the individual levels of ecological organization, using the "*varComp"* function (varcomp:ape). As explained in Messier et al. (2010), the variance component of a strata vc_i is defined by the estimated variance of this strata s_i^2 divided by the total variance among strata given as the sum of estimated variances of all strata.

$$vc_i = s_i^2 / \sum_{i=1}^{s} s_i^2$$

The code used in R to calculate the inter- and intra-specific variance of the trait SLA by computing the linear mixed-effect model as well as the variance components was:

(i):

```
varcompSLA_log_full_i_itv <-varcomp(lme(log(SLA_mean_ind) ~ 1 ,random
    = ~1|species, data = niche_all_ind, na.action = na.omit),TRUE)
```

The code for calculating the variance of the consecutive nested ecological scales used in R was:

(ii):

```
varcompSLA_log_g_i <-varcomp(lme(log(SLA_mean_ind) ~ 1 , random =
    ~1|glacier/transect/plot, data = niche_all_ind, na.action = na.omit,
    subset=species=="G"),TRUE)
```

The model for LNC and the other species was set up in analogous way.
It should be noted that the function "*varComp"* (varcomp:ape) will calculate a so-called within-level variance of the lowest organizational level, which represents the variation (i) within species plus the estimation error, or (ii) individuals within a plot plus the estimation error. I therefore call it (i) *ITV + error* and (ii) *Individuum + error* later.

Concerning assumptions for lme models I further note that there is no need for variance homogeneity in this calculation of variance components, as this requirement only refers to the derivation of accurate p-values (Zar, 1996).

Linear Mixed Effect Models

To analyse the variation of observed traits along abiotic and biotic environmental gradients, I built linear mixed-effect models (lme:nmle) for SLA and LNC data. In contrast to the models of the variance partitioning operations, where I aimed to gain insights into scale-dependent structuring of traits, I now added environmental predictors as fixed effects to detect responses of my focal traits to the environmental gradients. Because the environmental predictors were measured on plot scale, data analysis was done at the scale of plots. Therefore, I calculated plot mean values of both traits which I used in the following models.

I used predictors describing both the abiotic and the biotic environment. With respect to the abiotic environment, I combined indicators sampled in the field with indicators derived from the recorded species composition. The directly measured indicators comprise *Altitude* and exposition of the plot. Since exposition measured in degrees is a circular parameter, I transformed it to *Eastness*, given as the sine of the plot exposition, a parameter known to be correlated with micro-scale temperature and species richness in alpine terrain (Winkler et al., 2016). The derived descriptors of the abiotic environment included indicators of temperature (*lan_TEMP*), soil pH (*lan_REACT*), soil nutrient content (*lan_NUTRI*), and soil moisture (*lan_MOIST*). I calculated these indicators as cover weighted averages of indicator values of the species recorded in a plot according to the indicator value assignments of Landolt (2010). On the part of the biotic environment of the plots I used total plant cover (*Total cover*) and species richness (*Richness*) as predictors and indicators of the intensity of biotic interactions within a local community.

The random factor structure of the single-species models was defined analogous to the *variance component* analysis focusing on the variance of the consecutive nested ecological scales, model, species identity was included in the random factor structure, see below. A representative code used in R to compute the full (all species included) linear mixed-effect model of the plot-wise mean SLA is:

```
lme_SLAm_p_lan<-lme(log(SLA_mean_plot)  ~  cover_total*Richness+
    altitude+ Eastness+ lan_TEMP+ lan_REACT+ lan_NUTRI+ lan_MOIST,
    random= ~1|glacier/transect/plot/species, data= niche_p_sc)
```

The same logic applies to the trait LNC and to single-species models, where adjustments to the random factor to *random= ~1|glacier/transect/plot* were made in analogy to *variance component* analysis.

Analysis of ecological gradients and occurrence of traits

My expectations about the distribution of variance components across levels of ecological organization as well as about variation among plots rests on the assumption that both abiotic and biotic conditions vary between glacier forelands and transects. To check these assumptions, I calculated linear mixed-effect models relating *lan_TEMP*, *lan_REACT*, *lan_NUTRI*, and *lan_MOIST*, as well as *Total cover* and *Richness* to transect identity, i.e., the position of transects along the chronosequence, from the youngest to the oldest. To take into account the fact that some plots may host more than one target species and to avoid duplicates, random factors were set to *random= ~1|species/glacier*.

The code I used in R, with the example of *lan_TEMP*, was:

```
gradient_temp<-lme(lan_TEMP~transect,random=~1|species/glacier,
    data=niche_all)
```

The same logic applies for the other parameters analysed with this approach.

To check differences in the same four environmental indicators, *lan_TEMP*, *lan_REACT*, *lan_NUTRI*, and *lan_MOIST*, between the three glacier forelands I compared means of plot values in the three valleys by means of a Kruskal-Wallis Test. I further used a Wilcoxon test to analyse whether there are significant differences in the mean trait values among the three species.

Results

Traits and Ecological gradients

Both SLA and LNC differed significantly among the three species (Figure 2a & b), and these differences were correlated among the two traits. *Oxyria* had both highest SLA and LNC, and *Geum* the lowest mean value of both traits. *lan_TEMP*, *lan_NUTRI*, and *lan_MOIST* differed significantly among transects along the chronosequence (Figure 3). While *lan_TEMP* and *lan_NUTRI*, increased with age, *lan_MOIST* decreased with age. Mean indicator values of pH did not differ among transects.

At the level of the three glacier forelands, I found significant differences with respect to all four environmental descriptors tested. The only exception was soil moisture which was not significantly different between the Frosnitz- and Untersulzbach valley.

Similar to the abiotic environment, transects also differed significantly in their biotic descriptors (Figure 4). Both species numbers and total cover increased with distance from the glacier, i.e. with community age. While total plant cover varied significantly between all three valleys, species richness is similar in the Untersulzbach- and Viltragen valley, but lower in the Frosnitz valley.

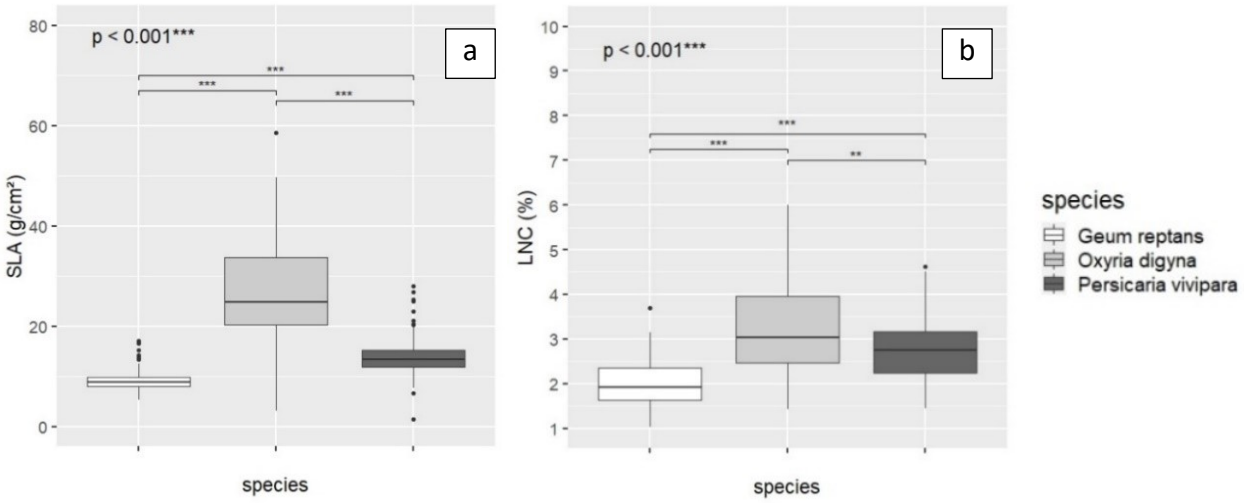

*Figure 2a & b: These figures show the expression and distribution of the morphological leaf trait SLA (a) and the leaf chemical trait LNC (b) of the three target species. The three different groups represent the target species Geum reptans (white), Oxyria digyna (grey) and Persicaria vivipara (dark grey). To test for differences between the three species Kruskal-Wallis test were performed. Additionally, pairwise Wilcox tests were conducted to analyze the difference between the single species. Indication for p-values: "***": p≤0.001, "**": p≤0.01, "*": p≤0.05, ".": p≤0.1.*

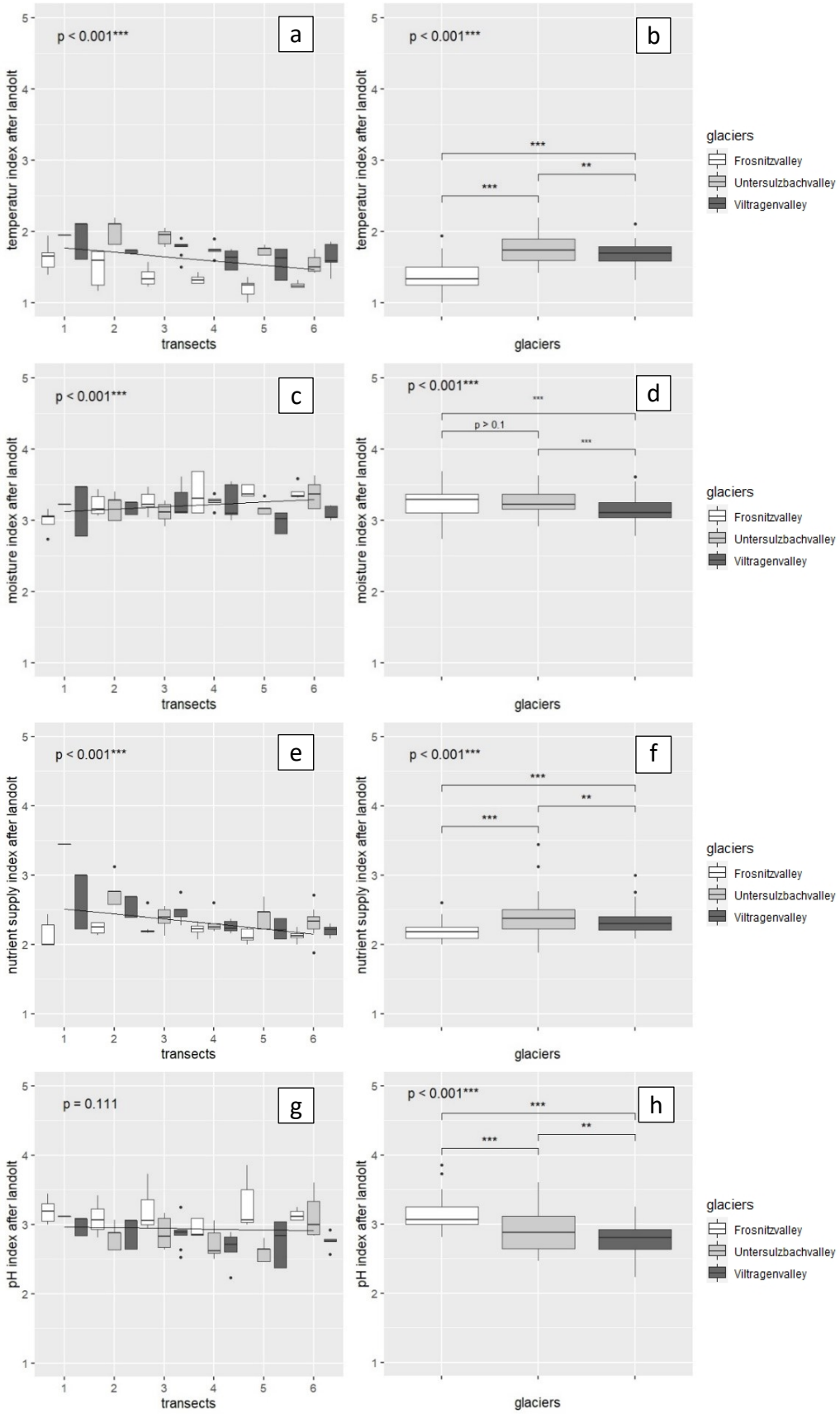

*Figure 3a-h: Figures show the change in abiotic environmental parameters temperature (a +b), moisture (c+d), nutrient supply (e+f) and pH (g+h) along an elevational gradient (left) as well as between the 3 glacier forelands (right). Boxplots show the distribution of the respective indices after Landolt et al. 2015 (left) within a given transect (1 being the oldest transect and 6 the youngest) & (right) within a glacial chronosequence of the (white) Frosnitz-, (grey) Untersulzbach- & (darkgrey) Viltragenvalley. Trendline & p-values derive from linear-mixed-effect-models. Kruskal-Wallis tests and pairwise Wilcox-test were performed to detect differences between datasets and groups. Indication for p-values: "***": p≤0.001, "**": p≤0.01, "*": p≤0.05, ".": p≤0.1.*

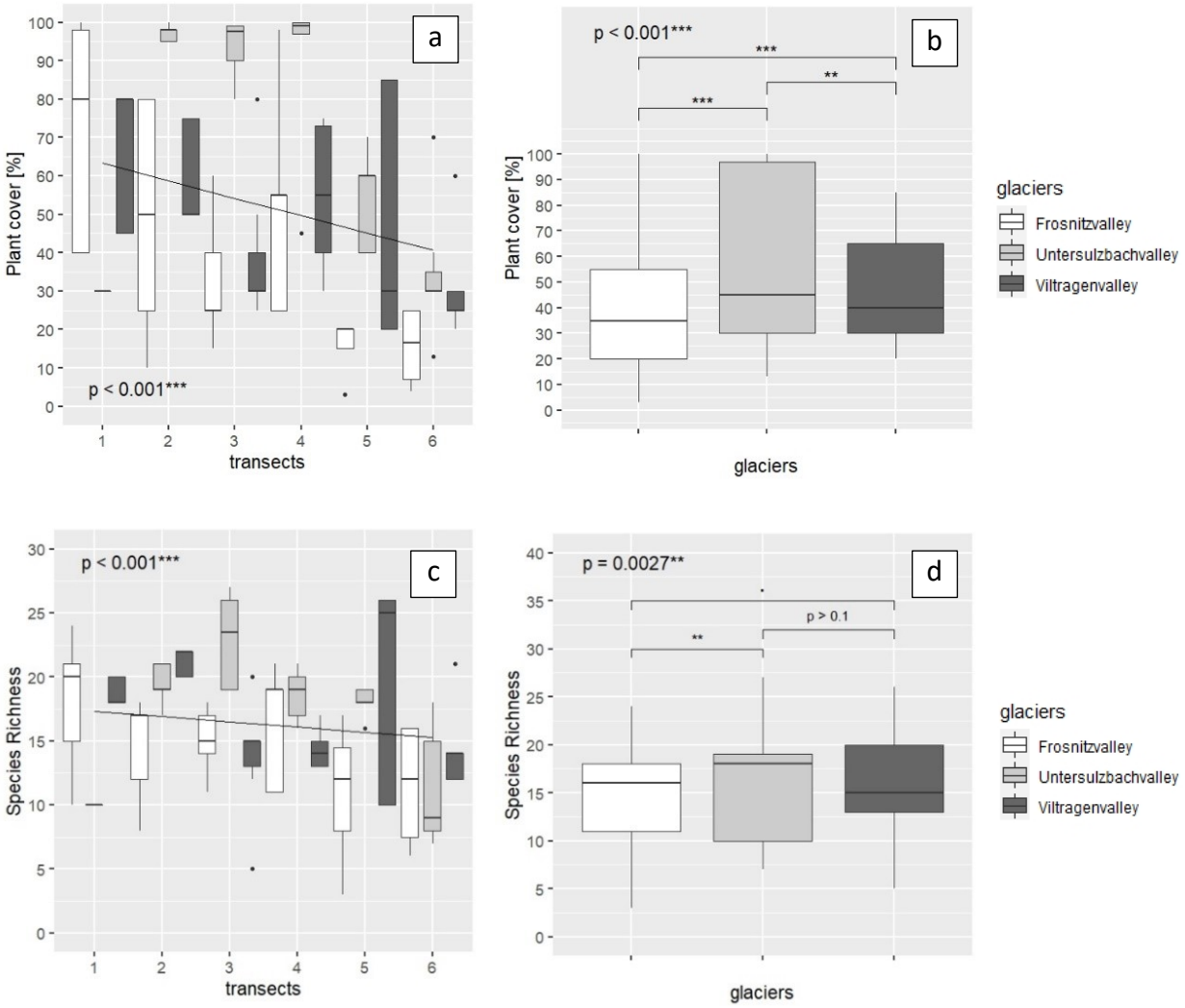

Figure *4a-d: Figures show the change in biotic parameters total plant cover (a+b) and Richness (c+d) along an elevational gradient (left side) & between the 3 glacier forelands (right side). Boxplots show the distribution of these proxies for concurrence (left side) within a given transect, starting from 1 being the oldest transect up to transect 6 which became ice-free 1-5 years ago & (right side) within a glacial chronosequence of the (white) Frosnitzvalley, (grey) Untersulzbachvalley & (darkgrey) Viltragenvalley. The trendline is the prediction of the linear-mixed-effect-model, p-values are indicating significance of models. For the boxplots a Kruskal-Wallis test was performed to detect if there was a significant difference between the data sets. Additionally, pairwise Wilcox tests were performed to analyze the difference between the groups. Indication for p-values: "***": p≤0.001, "**": p≤0.01, "*": p≤0.05, ".": p≤0.1.*

Table 1: Overview of the interspecific (species) and intraspecific (ITV + error) variation of the two traits SLA and LNC. The later partition includes the unexplained variance and has to be seen as ITV + error. Values are given in percentage of total variance across the two scales.

	SLA	**LNC**
species	85.83	43.53
ITV + error	14.17	56.47

Variance components and Intraspecific variance of traits

The analysis of the partition between inter- and intraspecific variation demonstrated salient differences among the two traits. While in case of SLA the variation attributed to different species identities accounted for 85.8% of the total variation, only 43.5% of the total variation was attributed to species level in the leaf chemical trait LNC (Table 1).

The further partitioning of the ITV also confirms the different variance distributions between the two different traits. While variation is concentrated between or within plots in case of SLA, the glacier level takes a far larger component of the variation in case of LNC. This partitioning is quite similar across all three species in case of LNC, but somewhat more variable in case of SLA (Table 2 & 3).

Table 2: Overview of SLA- variance partitioning, measured in percentage of total variance across different spatial scales and species.

	Geum	**Oxyria**	**Persicaria**
Glacier	1.69	6.57	14.57
Transect	7.58	0	13.22
Plot	21.56	67.38	29.58
Individuum + error	69.17	26.05	42.63

Table 3: Overview of LNC- variance partitioning, measured in percentage of total variance across different spatial scales and species.

	Geum	**Oxyria**	**Persicaria**
Glacier	52.4	34.1	48.3
Transect	4.3	12.82	1.37
Plot	21.25	28.38	27.2
Individuum + error	22.05	24.7	23.13

Mean trait variation along environmental gradients

The linear mixed-effects models suggest that SLA variation across all species was related to both abiotic and biotic conditions of the plots. Specific leaf area decreases with *Eastness* and *Total cover*, i.e. SLA was lower at places with high vegetation cover and those exposed more to the east; and it marginally increases with *lan_TEMP* and decreases with *Altitude.* SLA variation within species decreased with *lan_MOIST* in case of *Oxyria*, but increased with nutrient availability in

case of *Persicaria*. Variation in *Geum* did not respond to any of the gradients analysed, although the models suggest a marginally significant increase with *Eastness.*

In case of LNC, variation across all species was again negatively correlated with *Eastness*, i.e. leaf nitrogen concentration tended to be lower when plots were exposed more to the east, and it marginally increases with *lan_MOIST.* Individual species models showed only one significant relationship: the LNC of *Persicaria* increased with *Total cover of plots*.

*Table 4: Overview of lme-models resulting from mean SLA values. For a better overview, only parameters with a p-value ≤0.1 are listed in this table and significant parameters p≤0.05 are marked in bold. Full result-tables are available in supplementary materials. Indication for p-values: "***": p≤0.001, "**": p≤0.01, "*": p≤0.05, ".": p≤0.1.*

models:	predictor (p≤0.1):	p-values:	correlation:	goodness of fit: (marginal R^2/ conditional R^2)
all species	***Total cover***	**0.015 ***	-	0.250/ 0.999
	Eastness	**0.047 ***	-	
	lan_TEMP	0.073 .	+	
	Altitude	0.098 .	-	
Geum	*Eastness*	0.088 .	-	0.506/ 1
Oxyria	***lan_MOIST***	**0.013 ***	-	0.565/ 0.975
	Total cover:Richness	0.061 .	+	
	Eastness	0.072 .	+	
	lan_TEMP	0.087 .	+	
Persicaria	*Eastness*	0.055 .	-	0.748/ 0.983
	lan_NUTRI	0.064 .	+	

*Table 5: Overview of lme-models resulting from mean LNC values. For a better overview, only parameters with a p-value ≤0.1 are listed in this table and significant parameters p≤0.05 are marked in bold. Full result-tables are available in supplementary materials. Indication for p-values: "***": p≤0.001, "**": p≤0.01, "*": p≤0.05, ".": p≤0.1.*

models:	predictor (p≤0.1):	p-values:	correlation:	goodness of fit: (marginal R^2/ conditional R^2)
all species	***Eastness***	**0.036 ***	-	0.111 / 0.999
	lan_MOIST	0.098 .	+	
Geum	*N/A*	N/A	N/A	0.226/ 0.999
Oxyria	*lan_REACT*	0.073 .	+	0.356/ 0.963
Persicaria	***Total cover***	**0.036 ***	**+**	0.543/ 0.999

Discussion

The multi-layered approach to data analysis in this work delivers a wealth of information. In order to merge the different findings, I would like to discuss them from three different perspectives: (i) what is the relative importance of ITV in analysed traits, (ii) are niche-based filters the driving

forces behind species selection on glacier forelands and (iii) which environmental features determine mean species traits.

First, we can see striking difference between the two traits concerning the relative share between inter- and intraspecific variation of traits. This difference persists even after further partitioning of the ITV, so that in the case of LNC, most of the variation is attributed to the glacier level, while in case of SLA it appears concentrated at the plot and individuum level. One explanation of these patterns is that the separated populations in the three valleys show local adaptations, thereby increasing the ITV of LNC. Whether such adaptations are pheno- or genotypic, and to which extent they are correlated with peculiarities of abiotic and biotic environments is not known. Though, a relationship to abiotic environments appears at least plausible as nutrient availability also differed significantly among glacier forelands. Ordeñez et al. (2009) described in a global study about the relationship between leaf traits and soil measures, that LNC can be linked to soil total nitrogen. If this relationship holds not only for larger ecological scales, but also for the specific case of my research site, it is possible that trait variability occurs due to phenotypic plasticity in response to environmental differences. However, at the plot level no direct relationship between LNC trait values and the indicator value *Ian_NUTRI* could be found using plot-level lme models. To prove if relationships can be found using direct measures of total soil nitrogen instead of Landolt indicator values, plant available nitrogen in soils must be measured directly. Concerning my research question on the relative importance of inter- and intraspecific variability of traits, it can be said that ITV accounts for a non-negligible proportion in both traits SLA and LNC. Even though the measured ITV of SLA (14.2%) is lower than the global average of 25% determined by Siefert et al. (2015), the result of the chemical leaf trait LNC (56.5%) showed that ITV may even be more important than intraspecific variability in some cases. Therefore, my research supports previous findings that ITV should be considered when studying the relationship between environmental parameters and plant traits (Albert et al., 2010; Kichenin et al., 2013).

Second, my results demonstrate that biotic and abiotic environmental conditions significantly change along the glacial chronosequence. Against expectations, however, these patterns are not reflected in trait variation. In fact, a significant proportion of the variability is distributed between plots within transects rather than between transects. In addition to the plot level, the lowest organization scale, namely *Individual+error*, also exhibits a large proportion of the total variation, which is especially true for SLA. On this scale, the proportion of statistical noise cannot be determined more precisely, but it still provides important information. Taken together, these results suggest high trait divergence at smaller ecological levels of organization, which is also indicated by the weak relationships between traits and environmental parameters in the lme-models. This high divergence at sites of similar age can have various causes: (i) the presence of microsites within the 1x1m plots or so-called “safe sites” can make even 1 m² plots a heterogeneous environment (Blonder et al., 2018; Erschbamer et al., 2008; Opedal et al.,

2015; Raffl et al., 2006; Stark et al., 2017); (ii) stochastic processes can play a prominent role, particularly during early stages of succession on forelands (Bell, 2001; Hubbell, 2001) (Emerson & Gillespie, 2008). The latter would suggest a transition between a predominant importance of random colonization and establishment and of niche-based filtering with increasing age of the glacier foreland or (iii) that biotic interactions, as described by Chalmandrier et al. (2017) and de Bello et al. (2013) also play an important role at smaller ecological scales and therefore shape trait expression also under unfavourable environmental conditions. My conclusion from the results achieved is that I could not find sufficient evidence that niche filtering is the most important process for community assembly on glacier forelands. However, this does not imply that it may play an important role in the supposedly slow formation of alpine climax communities over time.

Third, I asked which features of plots determine mean trait values of species. In accordance with the limited evidence for niche filtering in the sampled communities I also found that the link between trait values and environmental conditions is only moderate. Which variables actually act as filters is hard to deduce from the data as glaciers and transects differ in environmental descriptors derived from the Landolt values, but these latter values are not or only marginally significant predictors of mean trait states. Nevertheless, the factor *Eastness* occurs in both cross-species models and in all single-species models of SLA. As shown by Winkler et al. (2016), east-exposed slopes in alpine grassland ecosystems are particularly rich in species due to the combined effect of high diurnal temperature sums and protection of westerly winds. I therefore expected traits to show a positive relationship with the environmental descriptor *Eastness*. However, the models showed that traits tended to respond in the opposite direction, with the exception of the SLA model of *Oxyria*. Especially in the early successional stages, glacier forelands consist mainly of bare ground, which Marcante et al. (2014) have shown, can lead to increased seedling mortality due to heat stress. From these results, it appears that *Eastness* rather is a proxy for drought stress due to high daily temperature sums during dry summer periods and thus act as stressor. Moreover, the mentioned case of *Oxyria* and the cross-species model of LNC show that *lan_MOIST* acts on traits in the opposite direction to *Eastness*, supporting the theory that drought might be a selective factor for trait variation in these glacier forelands with their often very shallow soils.

Taken together, this work highlights the importance of intraspecific variability for trait-based synecological questions. They also indicate that variation in SLA and LNC of the three target species in glacier foreland chronosequences is only moderately linked to environmental conditions, which can be particularly attributed to high trait divergence at smaller ecological levels of organization. There may be several reasons for the high variability of traits between and within plots of same age, such as the presence of favorable microhabitats, a high relevance of stochastic effects, insufficient time for niche filtering processes or biotic interactions. As a corollary, niche-

based processes apparently have a moderate impact on community assembly in this environment. From the results as they stand, drought stress (described by *Eastness* and *lan_MOIST*) is the most plausible predictor to explain mean trait differences, although this assumption is only weakly supported statistically.

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3. Schlussfolgerungen (Deutsch)

Diese Arbeit befasst sich mit der Frage, welche treibenden Faktoren die Zusammensetzungen von Pflanzengemeinschaften alpiner Sukzessionsflächen determinieren. Anhand der Analyse von Blatt-Merkmalen wird dabei versucht, die verschiedenen treibenden Kräfte sowie involvierten Prozesse zu identifizieren. Um ein möglichst vollständiges Bild zu erhalten, wird ein integrativer Ansatz verfolgt, bei dem sowohl inter- als auch intraspezifische Blatt-Merkmale über mehrere ökologische Skalenniveaus analysiert werden. Um die Ergebnisse der unterschiedlichen Modelle und Tests zusammenzuführen, möchte ich sie aus drei Blickwinkeln diskutieren und dabei auf meine Forschungsfragen eingehen: (i) Welche relative Bedeutung hat ITV in den untersuchten Blatt-Merkmalen, (ii) sind Nischen-basierte Filter die treibenden Kräfte hinter der Selektion von Arten auf Gletschervorfelder und (iii) welche Umweltfaktoren determinieren die Ausprägung von Blatt-Merkmalen.

Als erstes möchte ich auf die relative Bedeutung von inter- und intra-spezifischer Variabilität beider Blatt-Merkmale eingehen. Hier zeigt sich ein klarer Unterschied in der Varianz-Verteilung zwischen SLA und LNC. Während bei SLA 85,8% der Gesamtvariation auf Unterschiede zwischen Arten zurückzuführen sind, stellt dies für LNC mit 43,5% den kleineren Anteil dar. Unterschiede zwischen den beiden Merkmalen bleiben auch nach einer weiteren Aufteilung der ITV bestehen. So kann im Fall von LNC der größte Teil der Variation auf das ökologische Skalen-Niveau der Gletschervorfelder zurückgeführt werden, dies trifft für SLA jedoch nicht zu. Eine mögliche Erklärung für diese Muster ist die Ausbildung lokaler Subpopulationen durch die räumliche Distanz zwischen den Gletschervorfeldern. Durch Plastizität der Blatt-Merkmale zwischen Subpopulationen würde nicht nur die hohe Variation auf dem Niveau der Gletschervorfelder, sondern auch der hohe Anteil der ITV von LNC erklärt werden. Ob sich Subpopulationen nur durch phänotypische Anpassungen unterscheiden oder bereits genetisch distinkte Populationen gebildet haben, lässt sich ohne genetische Analyse nicht vollständig beantworten. Ein genauerer Blick auf die Analyse ökologischer Gradienten zeigt jedoch, dass sich auch die Nährstoffverfügbarkeit zwischen den Gletschervorfeldern erheblich unterscheidet. Ordeñez et al. (2009) hat in einer globalen Metastudie die Beziehung zwischen Blattmerkmalen und Bodenparametern untersucht und dabei einen Zusammenhang zwischen LNC und dem Gesamtstickstoffgehalt von Böden gefunden. Sollte dieser Zusammenhang nicht nur auf größeren Skala-Niveaus, sondern auch im impliziten Fall meines Untersuchungsgebietes gelten, so würde dies für phänotypische Anpassungen an unterschiedliche Habitatbedingungen sprechen. Betrachtet man zusätzlich die relevanten Umweltindikatoren der Aufnahmeflächen, so kann keine direkte Beziehung zwischen dem Blattstickstoffgehalt und dem Landoltwert *lan_NUTRI* festgestellt werden. Ob dieser Zusammenhang bei direkten Messungen des lokalen Bodenstickstoffgehalts anstelle der Indikatorwerte doch besteht, müsste jedoch in einer weiteren Studie geklärt werden.

In Bezug auf meine Forschungsfrage zur relativen Bedeutung der inter- und intraspezifischen Variabilität der Blatt-Merkmale kann gesagt werden, dass ITV eine nicht zu vernachlässigbare Größe bei der Untersuchung von Pflanzengemeinschaften darstellt. Auch wenn der gemessene ITV von SLA mit 14,2% niedriger ist als der von Siefert et al. (2015) ermittelte globale Durchschnitt von 25 %, so zeigte sich mit dem Ergebnis des chemischen Blatt-Merkmals LNC (56,5%), dass die ITV in einigen Fällen sogar wichtiger sein kann als die interspezifische Variabilität (Die Ergebnisse der ITV beinhalten einen statistischen Fehler-Anteil der nicht von dem expliziten Wert der intraspezifischen Variabilität getrennt werden kann. Deshalb sind die expliziten Werte der ITV kleiner als hier angegebenen. Siehe Manuskript-Teil). Die Ergebnisse meine Forschung unterstützen daher frühere Erkenntnisse, dass ITV bei der Untersuchung der Beziehung zwischen Umweltparametern und Pflanzenmerkmalen berücksichtigt werden sollte (Albert et al., 2010; Kichenin et al., 2013).

Zweitens zeigen die Ergebnisse, dass sich eine Reihe von biotischen und abiotischen Umweltparametern entlang der glazialen Chronosequenz signifikant ändern und somit den angenommen ökologischen Gradienten bestätigen. Entgegen der Erwartungen lässt sich dieser Gradient jedoch nicht in der Partitionierung der beiden Blatt-Merkmale erkennen. Ein bedeutender Faktor hierbei ist die Verteilung der Merkmalsvariabilität, welche zu einem großen Anteil zwischen den Aufnahmeflächen innerhalb der Transekte und nicht, wie erwartet, zwischen den Transekten zu finden ist. Neben der Variabilität zwischen Aufnahmeflächen eines Transekts, erklärt die kleinste gemessene ökologische Organisationseinheit, nämlich *Individuum+Fehler* innerhalb der Aufnahmeflächen, ebenso große Anteile der Gesamtvariation, dies gilt besonders für SLA. Zusammen genommen deuten diese Ergebnisse auf eine hohe Divergenz der Merkmale innerhalb kleinerer ökologischen Organisationseinheiten hin. Das Auftreten von relativ gesehen großer Variabilität der Blatt-Merkmale zwischen Flächen ähnlichen Alters wird durch die schwachen Zusammenhänge in den lme-Modellen der Habitat-Bedingungen unterstützt. Warum sowohl bei der Varianz-Partitionierung als auch den lme-Modellen keine starken Zusammenhänge zwischen Habitat-Indikatoren und Merkmalsausprägungen zu finden sind, kann verschiedene Ursachen haben: (i) so kann durch das Vorhandensein von Mikrohabitaten oder sogenannte "Safe Sites" innerhalb der 1x1m-Flächen die abiotischen Lebensraumbedingungen innerhalb einer Aufnahmefläche deutlich heterogener werden (Blonder et al., 2018; Erschbamer et al., 2008; Opedal et al., 2015; Raffl et al., 2006; Stark et al., 2017); oder (ii) stochastische Prozesse spielen in frühen Phasen der Besiedlung von Gletschervorfeldern eine größere Rolle als Selektion durch Habitatbedingungen bzw. haben Nischen-basierte Filter noch nicht genügend lange auf eine Pflanzengemeinschaft einwirken können um diese signifikant zu selektieren. Sollte dies zutreffen, so würde diese Erklärung einen Übergang von der Dominanz des Zufalls während der frühen Phasen der Besiedlung hin zu stärkerer Bedeutung ökologischer Filter (Emerson & Gillespie, 2008) für die Artenzusammensetzung mit zunehmendem Alter der Gletschervorfelder

nahe legen. Eine weitere mögliche Erklärung wäre, dass (iii) biotische Interaktionen, wie sie von Chalmandrier et al. (2017) und de Bello et al. (2013) beschrieben werden, besonders auf kleineren ökologischen Skalen in Form von Konkurrenz auf Individuen einwirken und somit die Divergenz an Merkmalsausprägungen auch unter ungünstigen Umweltbedingungen fördern. Zusammenfassend schließe ich aus meinen Ergebnissen, dass Nischen-basierte Prozesse nicht als einzige und primär selektierende Faktoren auf Gletschersukzessionsflächen identifiziert werden können. Da verschiedene Erklärungsmöglichkeiten für die Ergebnisse bestehen, ist es notwendig an diesem Punkt weitere Forschung anzuschließen.

Drittens, beschäftigt sich meine letzte Forschungsfrage mit der Thematik, welche Umweltfaktoren die Ausprägung von Blatt-Merkmalen determinieren. Wie bereits beschrieben, habe ich entgegen meinen Erwartungen nur einen geringen Zusammenhang zwischen Merkmalsausprägungen und Habitatbedingungen der Aufnahmeflächen gefunden. Welche Variablen tatsächlich als Filter wirken, lässt sich aus meinen Daten nicht vollständig beantworten. Dennoch zeichnen sich gewisse Tendenzen ab, an denen weitere Forschung anknüpfen kann. Hier ist besonders der Faktor *Eastness* zu erwähnen. Wie Winkler et al. (2016) gezeigt haben, sind Ost-exponierte Hänge in alpinen Rasen aufgrund der kombinierten Wirkung hoher Tagestemperatursummen und dem Schutz vor Westwinden besonders artenreich. Basierend auf dieser Studie hatte ich erwartet, dass Blatt-Merkmale einen positiven Zusammenhang mit dem Umweltdeskriptor *Eastness* aufweisen würden. Die lme-Modelle zeigten jedoch, dass beide Blatt-Merkmale tendenziell in die entgegengesetzte Richtung reagierten, einzige Ausnahme war hier das SLA-Modell für *Oxyria*. Vor allem in den frühen Sukzessionsstadien bestehen die Böden von Gletschervorfeldern aus Schotter, Sand und Schluff. Laut Marcante et al. (2014) unterliegen Keimlinge auf diesen Rohböden, die eine geringe Wasserhaltekapazität aufweisen, einer erhöhten Sterblichkeit durch Hitzestress in den heißen Sommermonaten. Betrachtet man meine Ergebnisse nun aus diesem Gesichtspunkt, so lässt sich vermuten, dass der Faktor *Eastness* auf Gletschervorfeldern durch die hohen täglichen Temperatursummen im Sommer eher als Indikator von Trockenstress gesehen werden kann. Der erwähnte Ausnahmefall von *Oxyria* und das artenübergreifende Modell von LNC, zeigen, dass der Feuchtigkeitsindikator *Ian_MOIST* in entgegengesetzter Richtung zu *Eastness* auf die Blattmerkmale einwirkt. Dieses Ergebnis stützt die Theorie, dass Trockenstress ein selektiver Faktor für die Ausprägung von Blatt-Merkmalen, insbesondere SLA, ist.

Als Resümee lässt sich sagen, dass ein mäßiger Zusammenhang zwischen den untersuchten Blattmerkmalen und Umweltindikatoren besteht. Beide Merkmale weisen zusätzlich eine hohe Merkmalsdivergenz auf kleinen ökologischen Organisationsskalen zwischen und innerhalb der Aufnahmeflächen auf. Mögliche Erklärungsmodellen dafür sind das Vorhandensein von günstigen Mikrohabitaten, eine hohe Relevanz stochastischer Effekte während früher Besiedlungsprozesse, unzureichend Zeit für Nischen-basierte Selektion oder Relevanz biotischer

Interaktion. Folglich kann die Zusammensetzung von Pflanzengemeinschaften der untersuchten Gletschervorfelder nicht primär durch Nischen-basierte Selektion erklärt werden. Auf dem Skalenniveau der einzelnen Aufnahmeflächen konnte Trockenstress (beschrieben durch *Eastness* und *lan_MOIST*) als plausibelster Prädiktor für Variabilität der Blatt-Merkmale identifiziert werden, statistisch wird diese Annahme jedoch nur schwach gestützt. Insbesondere durch die Ergebnisse des chemischen Blattmerkmal LNC, bei dem ITV mehr als 50% der Gesamtvariation ausmacht, wird mit dieser Arbeit die Bedeutung der intraspezifischen Variabilität für synökologische Fragestellungen hervorgehoben.

4. Supplementary Material

Full lme-tables

SLA

*table 6: Overview of full lme-models resulting from mean SLA values. Indication for p-values: "***": p≤0.001, "**": p≤0.01, "*": p≤0.05, ".": p≤0.1. Significant parameters p≤0.05 are marked in bold.*

models:	predictor (p≤0.1):	p-values:	correlation:	goodness of fit: (marginal R^2/ conditional R^2)
	Total cover	**0.015 ***	-	
	Richness	0.142	-	
	Altitude	0.098 .	-	
	Eastness	**0.046 ***	-	
all species	*lan_TEMP*	0.071	+	0.250/ 0.999
	lan_REACT	0.386	+	
	lan_NUTRI	0.587	+	
	lan_MOIST	0.175	+	
	Total cover:Richness	0.382	-	
	Total cover	0.364	-	
	Richness	0.145	-	
	Altitude	0.133	+	
	Eastness	0.088 .	-	
Geum	*lan_TEMP*	0.205	+	0.506/ 1
	lan_REACT	0.272	-	
	lan_NUTRI	0.243	-	
	lan_MOIST	0.877	-	
	Total cover:Richness	0.198	-	
	Total cover	0.361	-	
	Richness	0.622	-	
	Altitude	0.108	+	
	Eastness	0.072 .	+	
Oxyria	*lan_TEMP*	0.087 .	+	0.565/ 0.975
	lan_REACT	0.465	-	
	lan_NUTRI	0.230	+	
	lan_MOIST	0.013 *	-	
	Total cover:Richness	0.061 .	+	
	Total cover	0.429	+	
	Richness	0.240	+	
	Altitude	0.778	-	
	Eastness	0.055 .	-	
Persicaria	*lan_TEMP*	0.947	-	0.748/ 0.983
	lan_REACT	0.241	-	
	lan_NUTRI	0.064 .	+	
	lan_MOIST	0.791	-	
	Total cover:Richness	0.168	-	

LNC

*table 7: Overview of full lme-models resulting from mean LNC values. Indication for p-values: "***": p≤0.001, "**": p≤0.01, "*": p≤0.05, ".": p≤0.1. Significant parameters p≤0.05 are marked in bold.*

models:	predictor (p≤0.1):	p-values:	correlation:	goodness of fit: (marginal R^2/ conditional R^2)
	Total cover	0.677	**+**	
	Richness	0.994	**+**	
	Altitude	0.545	**-**	
	Eastness	0.036 *	**-**	
all species	*lan_TEMP*	0.225	**+**	0.111 / 0.999
	lan_REACT	0.105	**+**	
	lan_NUTRI	0.539	**-**	
	lan_MOIST	0.098 .	**+**	
	Total cover:Richness	0.765	**-**	
	Total cover	0.565	+	
	Richness	0.859	-	
	Altitude	0.283	+	
	Eastness	0.114	-	
Geum	*lan_TEMP*	0.820	+	0.226/ 0.999
	lan_REACT	0.353	-	
	lan_NUTRI	0.133	-	
	lan_MOIST	0.639	+	
	Total cover:Richness	0.999	+	
	Total cover	0.741	-	
	Richness	0.116	+	
	Altitude	0.494	+	
	Eastness	0.786	-	
Oxyria	*lan_TEMP*	0.220	+	0.356/ 0.963
	lan_REACT	0.073 .	+	
	lan_NUTRI	0.361	-	
	lan_MOIST	0.458	+	
	Total cover:Richness	0.326	+	
	Total cover	0.036 *	+	
	Richness	0.140	+	
	Altitude	0.289	+	
	Eastness	0.557	-	
Persicaria	*lan_TEMP*	0.575	+	0.543/ 0.999
	lan_REACT	0.870	-	
	lan_NUTRI	0.181	+	
	lan_MOIST	0.537	-	
	Total cover:Richness	0.104	-	

R-Code

```
########################
### Masterarbeit ITV ###
########################

###############
### setups: ###
###############

### Install packages
## for lme & distribution partitioning

install.packages("nlme") #lme
library(nlme)
install.packages("ape") #varcomp
library(ape)
install.packages("piecewiseSEM") #rsquared
library(piecewiseSEM)
install.packages("ggpubr")  #compare_mean
library("ggpubr")
install.packages("ggplot2") #plots
library("ggplot2")

### setting working directory
setwd("C:/Users/marlo/Dokumente/marlon/uni/Masterthesis Intra-species trait
variability/R")

#================================================#

##################
### load data: ###
##################

### Load file:
traits<-read.csv("C:/Users/marlo/Dokumente/marlon/uni/Masterthesis Intra-species trait
variability/R/trait_all.csv")
cn_all<-read.csv("C:/Users/marlo/Dokumente/marlon/uni/Masterthesis Intra-species trait
variability/CN/CN_all.csv")
niche_all<-read.csv("C:/Users/marlo/Dokumente/marlon/uni/Masterthesis Intra-species
trait variability/R/niche_all.csv")

#================================================#

############################
### preparation of data: ###
############################

### convert exposition(circular parameter) into eastness and northness
niche_all<-
cbind(niche_all,Eastness=(sin(niche_all$aspect)),Northness=cos(niche_all$aspect))

## aggregate data to individuum niveau
SLA_mean<-aggregate(SLA~Nr_plant+species, FUN=mean, data=niche_all)
niche_all_ind<-
aggregate(niche_all[,c(3,4,5,7,9,10,11,12,13,14,15,19,20,21,22,23,24,25,26,27)], by =
list(Nr_plant=niche_all$Nr_plant, species=niche_all$species), FUN=mean,na.rm=TRUE)
cn_all<-cn_all[order(cn_all$species,cn_all$Nr_plant),]
niche_all_ind<-niche_all_ind[order(niche_all_ind$species, niche_all_ind$Nr_plant),]
niche_all_ind<-cbind(niche_all_ind,cn_all[,c(4,5,9,10,11,12)])
colnames(niche_all_ind)[14]<-("SLA_mean_ind")

### attach C:LNC ratio
niche_all_ind<-transform(niche_all_ind, CN= LCC/LNC)

### reduce Ranunculus
traits<-traits[traits$species!="R",]
cn_all<-cn_all[cn_all$species!="R",]
niche_all<-niche_all[niche_all$species!="R",]
```

```
### reduce extra plots # because the cn_all file does not contain the transect
information date have to be aggregated first and then be reduced afterwards
traits<-traits[traits$transect!=0,]
niche_all<-niche_all[niche_all$transect!=0,]

### reduce individuum niveau
niche_all_ind<-niche_all_ind[niche_all_ind$species!="R",]
niche_all_ind<-niche_all_ind[niche_all_ind$transect!=0,]

#================================================#

############################################################
### Variance of traits on different ecological scales: ###
############################################################

#---------------------------#
# Variance Componence -SLA  #
#---------------------------#
#-------------------------------------------------#
# Variance Componence -SLA until individuum niveau  #
#-------------------------------------------------#
### full calculations
### Result is a vector with variance components at the respective levels. The last
level is "within" and refers to the level of leaves in case of SLA / to the level of
individuals in case of LNC and CN ratio.
### model calculation inter- & intra-specific variability of traits
varcompSLA_log_full_i_itv <-varcomp(lme(log(SLA_mean_ind) ~ 1 ,random = ~1|species,
data = niche_all_ind, na.action = na.omit),TRUE)

#-----------------------------------------------------------------#
# Variance Componence - SLA Geum only - until individuum niveau #
#-----------------------------------------------------------------#
### Geum only
## Variance component model
# log-transformed
varcompSLA_log_g_i <-varcomp(lme(log(SLA_mean_ind) ~ 1 ,random =
~1|glacier/transect/plot, data = niche_all_ind, na.action = na.omit,
subset=species=="G"),TRUE)
#-------------------------------------------------#

#-------------------------------------------------------------------#
# Variance Componence - SLA Persicaria only - until individuum niveau #
#-------------------------------------------------------------------#
varcompSLA_log_p_i <-varcomp(lme(log(SLA_mean_ind) ~ 1 ,random =
~1|glacier/transect/plot, data = niche_all_ind, na.action = na.omit,
subset=species=="P"),TRUE)
#-------------------------------------------------#

#-----------------------------------------------------------------#
# Variance Componence - SLA Oxyria only - until individuum niveau #
#-----------------------------------------------------------------#
varcompSLA_log_o_i <-varcomp(lme(log(SLA_mean_ind) ~ 1 ,random =
~1|glacier/transect/plot, data = niche_all_ind, na.action = na.omit,
subset=species=="O"),TRUE)
#-------------------------------------------------#

#-----------------------------------------------------------#
# Variance Componence - overview SLA - until individuum niveau #
#-----------------------------------------------------------#
varpar_i<-cbind(varcompSLA_log_g_i, varcompSLA_log_p_i, varcompSLA_log_o_i,
varcompSLA_log_full_i[-4])
varpar_i<-rbind(varpar_i[1:3,],NA,varpar_i[4,])
varpar_i[4,]<- c(NA,NA,NA,varcompSLA_log_full_i[4])
colnames(varpar_i)<- c("Geum","Persicaria", "Oxyria", "all")
rownames(varpar_i)[4]<-"species"
rownames(varpar_i)[5]<-"individuum+error"
varpar_i<-varpar_i*100
varpar_i<-format(round(varpar_i, 2), scientific =FALSE)
write.csv(varpar_i, "variance_partitioning_SLA_all_data_individuum.csv")
#-------------------------------------------------#
```

```
#--------------------------#
# Variance Componence -LNC  #
#--------------------------#
#---------------------------------------#
# Variance Componence -LNC full data-set #
#---------------------------------------#
### full calculations
### model calculation inter- & intra-specific variability of traits
varcomp_N_log_full_itv <-varcomp(lme(log(LNC) ~ 1 ,random = ~1|species, data =
niche_all_ind, na.action = na.omit),TRUE)

#-------------------------------------#
# Variance Componence - LNC Geum only #
#-------------------------------------#
### Geum only
## Variance component model
# log-transformed
varcomp_N_log_g <-varcomp(lme((log(LNC)) ~ 1 ,random = ~1|glacier/transect/plot, data
= niche_all_ind, na.action = na.omit, subset=species=="G"),TRUE)
qqnorm(resid(lme((log(LNC)) ~ 1 ,random = ~1|glacier/transect/plot, data =
niche_all_ind, na.action = na.omit, subset=species=="G")))
qqline(resid(lme((log(LNC)) ~ 1 ,random = ~1|glacier/transect/plot, data =
niche_all_ind, na.action = na.omit, subset=species=="G")))
#-----------------------------------------------------#

#---------------------------------------#
# Variance Componence - LNC Persicaria only #
#---------------------------------------#
### Persicaria only
## Variance component model
# log-transformed
varcomp_N_log_p <-varcomp(lme((log(LNC)) ~ 1 ,random = ~1|glacier/transect/plot, data
= niche_all_ind, na.action = na.omit, subset=species=="P"),TRUE)
qqnorm(resid(lme((log(LNC)) ~ 1 ,random = ~1|glacier/transect/plot, data =
niche_all_ind, na.action = na.omit, subset=species=="P")))
qqline(resid(lme((log(LNC)) ~ 1 ,random = ~1|glacier/transect/plot, data =
niche_all_ind, na.action = na.omit, subset=species=="P")))
#-----------------------------------------------------#

#-------------------------------------#
# Variance Componence - LNC Oxyria only #
#-------------------------------------#
### Persicaria only
## Variance component model
# log-transformed
varcomp_N_log_o <-varcomp(lme(log(LNC) ~ 1 ,random = ~1|glacier/transect/plot, data =
niche_all_ind, na.action = na.omit, subset=species=="O"),TRUE)
qqnorm(resid(lme((log(LNC)) ~ 1 ,random = ~1|glacier/transect/plot, data =
niche_all_ind, na.action = na.omit, subset=species=="O")))
qqline(resid(lme((log(LNC)) ~ 1 ,random = ~1|glacier/transect/plot, data =
niche_all_ind, na.action = na.omit, subset=species=="O")))
#-----------------------------------------------------#

#-------------------------------------------------------------#
# Variance Componence - overview LNC until individuum niveau #
#-------------------------------------------------------------#
varpar_LNC<-cbind(varcomp_N_log_g, varcomp_N_log_p, varcomp_N_log_o,
varcomp_N_log_full[-4])
varpar_LNC<-rbind(varpar_LNC[1:3,],NA,varpar_LNC[4,])
varpar_LNC[4,]<- c(NA,NA,NA,varcomp_N_log_full[4])
colnames(varpar_LNC)<- c("Geum","Persicaria", "Oxyria", "all")
rownames(varpar_LNC)[4]<-"species"
rownames(varpar_LNC)[5]<-"individuum+error"
varpar_LNC<-varpar_LNC*100
varpar_LNC<-format(round(varpar_LNC, 2), scientific =FALSE)
write.csv(varpar_LNC, "variance_partitioning_LNC_all_data.csv")
#-----------------------------------------------#
#===============================================#
```

```
########################
### plotting traits: ###
########################

## ggplot - SLA~species
#check assumptions for Comparison between LNC of species
x11()
qqnorm(traits$SLA)
qqline(traits$SLA)
shapiro.test(traits$SLA) #non normal -->Kruskal-Wallis Test
kruskal.test(SLA~species, data=traits)
pairwise.wilcox.test(traits$SLA,traits$species, p.adjust.method = "bonferroni")
my_comparisons <- list( c("1", "2"), c("2", "3"), c("1", "3") )
pwc_LNC<-compare_means(SLA~species,data= traits,comparison=my_comparisons, method =
"wilcox.test",p.adjust.method = "bonferroni")

# custom graph because p.adj /= p.signif
df1 <- data.frame(a = c(1, 1:3,3), b = c(69, 70, 70,70, 69))
df2 <- data.frame(a = c(1, 1,2, 2), b = c(66, 67, 67, 66))
df3 <- data.frame(a = c(2, 2, 3, 3), b = c(64, 65, 65, 64))

# boxplot
x11()
ggplot(data = traits,aes(x=factor(species), y=SLA)) +
  geom_boxplot(position=position_dodge(1), show.legend = T,
aes(fill=factor(species)))+
  scale_fill_manual(values=c("white", "grey80", "grey40"), name="species",
labels=c("Geum reptans", "Oxyria digyna", "Persicaria vivipara"))+
  scale_y_continuous(limits=c(0,80))+
  scale_x_discrete(labels=c("","",""))+
  theme_gray(base_size = 17) +
  xlab("species") +
  ylab("SLA (g/cm)")+
  geom_line(data = df1, aes(x = a, y = b)) + annotate("text", x = 2, y = 71, label =
"***", size = 4.5) +
  geom_line(data = df2, aes(x = a, y = b)) + annotate("text", x = 1.5, y = 68, label =
"***", size = 4.5) +
  geom_line(data = df3, aes(x = a, y = b)) + annotate("text", x = 2.5, y = 66, label =
"***", size =4.5)+
  annotate("text", x = 1, y = 78, label = "  p < 0.001***",size=6 )

## ggplot - LNC~species
#check assumptions for Comparison between LNC of species
x11()
qqnorm(cn_all$LNC)
qqline(cn_all$LNC)
shapiro.test(cn_all$LNC) #non normal -->Kruskal-Wallis Test
kruskal.test(LNC~species, data=cn_all)
pairwise.wilcox.test(cn_all$LNC,cn_all$species, p.adjust.method = "bonferroni")
my_comparisons <- list( c("1", "2"), c("2", "3"), c("1", "3") )
pwc_LNC<-compare_means(LNC~species,data= cn_all,comparison=my_comparisons, method =
"wilcox.test",p.adjust.method = "bonferroni")

# custom graph because p.adj /= p.signif
df1 <- data.frame(a = c(1, 1:3,3), b = c(7.5, 7.6, 7.6,7.6,7.5))
df2 <- data.frame(a = c(1, 1,2, 2), b = c(7.15, 7.25, 7.25, 7.15))
df3 <- data.frame(a = c(2, 2, 3, 3), b = c(6.9, 7, 7, 6.9))

# boxplot
x11()
ggplot(data = cn_all,aes(x=factor(species), y=LNC)) +
  geom_boxplot(position=position_dodge(1), show.legend = F,
aes(fill=factor(species)))+
  scale_fill_manual(values=c("white", "grey80", "grey40"), name="species",
labels=c("Geum reptans", "Oxyria digyna", "Persicaria vivipara"))+
  scale_y_continuous(limits=c(1,10), breaks =c(1,2,3,4,5,6,7,8,9,10))+
  scale_x_discrete(labels=c("","",""))+
  theme_gray(base_size = 17)+
  xlab("species") +
  ylab("LNC (%)")+
```

```
  geom_line(data = df1, aes(x = a, y = b)) + annotate("text", x = 2, y = 7.8, label =
"***", size = 4.5) +
  geom_line(data = df2, aes(x = a, y = b)) + annotate("text", x = 1.5, y = 7.35, label
= "***", size = 4.5) +
  geom_line(data = df3, aes(x = a, y = b)) + annotate("text", x = 2.5, y = 7.1, label
= "**", size =4.5)+
  annotate("text", x = 1, y = 9.5, label = "  p < 0.001***",size=6 )

#=================================================#

################################
### lme's individuum niveau: ###
################################

### scale data
niche_all_sc<-scale(niche_all_ind[,7:13],center = TRUE, scale = TRUE)
niche_all_sc<-cbind(niche_all_sc, scale(niche_all_ind[,15:22],center = TRUE, scale =
TRUE))
niche_all_sc<-
cbind(niche_all_ind[,c(23,1,2,3,4,5,6)],niche_all_sc,niche_all_ind[,c(14,25,26,27)])

#-------------#
# all species #
#-------------#

##---------------------------#
## SLA mean-> shift of niche #
##---------------------------#

## Interaction-Model with landolt values
lme_SLAm_ind_lan<-lme(log(SLA_mean_ind) ~
cover_total*Richness+altitude+Eastness+lan_TEMP+lan_REACT+lan_NUTRI+lan_MOIST, random=
~1|glacier/transect/plot/species/individuum, data= niche_all_sc)
summary(lme_SLAm_ind_lan)

#check assumtions:
x11()
qqnorm(resid(lme_SLAm_ind_lan))
qqline(resid(lme_SLAm_ind_lan))

## Pseudo R^2 -lme
rsquared(lme_SLAm_ind_lan)

##----------#
## LNC mean #
##----------#

## Interaction-Model with landolt values
lme_LNCm_ind_lan<-lme(log(LNC) ~
cover_total*Richness+altitude+Eastness+lan_TEMP+lan_REACT+lan_NUTRI+lan_MOIST, random=
~1|glacier/transect/plot/species/individuum, data= niche_all_sc)
summary(lme_LNCm_ind_lan)

#check assumtions:
x11()
qqnorm(resid(lme_LNCm_ind_lan))
qqline(resid(lme_LNCm_ind_lan))

## Pseudo R^2 -lme
rsquared(lme_LNCm_ind_lan)

#-----------------------#
# single species models #
#-----------------------#
#-------------------#
# Geum reptans only #
#-------------------#
##----------#
## SLA mean #
##----------#
```

```
### linear mixed effect model
## Interaction-Model with landolt values
lme_SLAm_g_ind_lan<-lme(log(SLA_mean_ind) ~
cover_total*Richness+altitude+Eastness+lan_TEMP+lan_REACT+lan_NUTRI+lan_MOIST, random=
~1|glacier/transect/plot/individuum, data= niche_all_sc, subset=species=="G")
summary(lme_SLAm_g_ind_lan)

#check assumtions:
x11()
qqnorm(resid(lme_SLAm_g_ind_lan))
qqline(resid(lme_SLAm_g_ind_lan))

## Pseudo R^2 -lme
rsquared(lme_SLAm_g_ind_lan)

##----------#
## LNC mean #
##----------#

#check normal distribution:
x11()
qqnorm(log(niche_all_sc$LNC)[niche_all_sc$species=="G"])
qqline(log(niche_all_sc$LNC)[niche_all_sc$species=="G"])
shapiro.test(log(niche_all_sc$LNC)[niche_all_sc$species=="G"])
#ND

### linear mixed effect model
## Interaction-Model with landolt values
lme_LNCm_g_ind_lan<-lme(log(LNC) ~
cover_total*Richness+altitude+Eastness+lan_TEMP+lan_REACT+lan_NUTRI+lan_MOIST, random=
~1|glacier/transect/plot/individuum, data= niche_all_sc, subset=species=="G")
summary(lme_LNCm_g_ind_lan)

#check assumtions:
x11()
qqnorm(resid(lme_LNCm_g_ind_lan))
qqline(resid(lme_LNCm_g_ind_lan))

## Pseudo R^2 -lme
rsquared(lme_LNCm_g_ind_lan)

#-------------#
# Oxyria only #
#-------------#
##----------#
## SLA mean #
##----------#

## Interaction-Model with landolt values
lme_SLAm_o_ind_lan<-lme(log(SLA_mean_ind) ~
cover_total*Richness+altitude+Eastness+lan_TEMP+lan_REACT+lan_NUTRI+lan_MOIST, random=
~1|glacier/transect/plot/individuum, data= niche_all_sc, subset=species=="O")
summary(lme_SLAm_o_ind_lan)

#check assumtions:
x11()
qqnorm(resid(lme_SLAm_o_ind_lan))
qqline(resid(lme_SLAm_o_ind_lan))

## Pseudo R^2 -lme
rsquared(lme_SLAm_o_ind_lan)


##----------#
## LNC mean #
##----------#

### linear mixed effect model
## Interaction-Model with landolt values
```

```
lme_LNCm_o_ind_lan<-lme(log(LNC) ~
cover_total*Richness+altitude+Eastness+lan_TEMP+lan_REACT+lan_NUTRI+lan_MOIST, random=
~1|glacier/transect/plot/individuum, data= niche_all_sc, subset=species=="O")
summary(lme_LNCm_o_ind_lan)

#check assumtions:
x11()
qqnorm(resid(lme_LNCm_o_ind_lan))
qqline(resid(lme_LNCm_o_ind_lan))

## Pseudo R^2 -lme
rsquared(lme_LNCm_o_ind_lan)

#-----------------#
# Persicaria only #
#-----------------#
##----------#
## SLA mean #
##----------#

### linear mixed effect model
## Interaction-Model with landolt values
lme_SLAm_p_ind_lan<-lme(log(SLA_mean_ind) ~
cover_total*Richness+altitude+Eastness+lan_TEMP+lan_REACT+lan_NUTRI+lan_MOIST, random=
~1|glacier/transect/plot/individuum, data= niche_all_sc, subset=species=="P")
summary(lme_SLAm_p_ind_lan)

#check assumtions:
x11()
qqnorm(resid(lme_SLAm_p_ind_lan))
qqline(resid(lme_SLAm_p_ind_lan))

## Pseudo R^2 -lme
rsquared(lme_SLAm_p_ind_lan)

##----------#
## LNC mean #
##----------#

### linear mixed effect model
## Interaction-Model with landolt values
lme_LNCm_p_ind_lan<-lme(log(LNC) ~
cover_total*Richness+altitude+Eastness+lan_TEMP+lan_REACT+lan_NUTRI+lan_MOIST, random=
~1|glacier/transect/plot/individuum, data= niche_all_sc, subset=species=="P")
summary(lme_LNCm_p_ind_lan)

#check assumtions:
x11()
qqnorm(resid(lme_LNCm_p_ind_lan))
qqline(resid(lme_LNCm_p_ind_lan))

## Pseudo R^2 -lme
rsquared(lme_LNCm_p_ind_lan)

##########################
### lme's plot niveau: ###
##########################

### aggregate data to plot niveau
niche_all_plot<-
aggregate(niche_all_ind[,c(3,4,5,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,26,27,28
,29)], by = list(NR_plot=niche_all_ind$plots, species=niche_all_ind$species),
FUN=mean,na.rm=TRUE)
colnames(niche_all_plot)[13]<-("SLA_mean_plot")

## scale data
niche_p_sc<-scale(niche_all_plot[,6:12],center = TRUE, scale = TRUE)
niche_p_sc<-cbind(niche_p_sc, scale(niche_all_plot[,14:21],center = TRUE, scale =
TRUE))
```

```
niche_p_sc<-cbind(niche_all_plot[,c(1:5)], niche_p_sc,
SLA_mean_plot=niche_all_plot[,13], niche_all_plot[,(22:25)])

##---------------------------#
## SLA mean-> shift of niche #
##---------------------------#

## Interaction-Model with landolt values
lme_SLAm_p_lan<-lme(log(SLA_mean_plot) ~
cover_total*Richness+altitude+Eastness+lan_TEMP+lan_REACT+lan_NUTRI+lan_MOIST, random=
~1|glacier/transect/plot/species, data= niche_p_sc)
summary(lme_SLAm_p_lan)

#check assumtions:
x11()
qqnorm(resid(lme_SLAm_p_lan))
qqline(resid(lme_SLAm_p_lan))

## Pseudo R^2 -lme
rsquared(lme_SLAm_p_lan)

##----------#
## LNC mean #
##----------#

## Interaction-Model with landolt values
lme_LNCm_p_lan<-lme(log(LNC) ~
cover_total*Richness+altitude+Eastness+lan_TEMP+lan_REACT+lan_NUTRI+lan_MOIST, random=
~1|glacier/transect/plot/species, data= niche_p_sc)
summary(lme_LNCm_p_lan)

#check assumtions:
x11()
qqnorm(resid(lme_LNCm_p_lan))
qqline(resid(lme_LNCm_p_lan))

## Pseudo R^2 -lme
rsquared(lme_LNCm_p_lan)

#-----------------------#
# single species models #
#-----------------------#
#-------------------#
# Geum reptans only #
#-------------------#
##----------#
## SLA mean #
##----------#

### linear mixed effect model
## Interaction-Model with landolt values
lme_SLAm_g_p_lan<-lme(log(SLA_mean_plot) ~
cover_total*Richness+altitude+Eastness+lan_TEMP+lan_REACT+lan_NUTRI+lan_MOIST, random=
~1|glacier/transect/plot, data= niche_p_sc, subset=species=="G")
summary(lme_SLAm_g_p_lan)

#check assumtions:
x11()
qqnorm(resid(lme_SLAm_g_p_lan))
qqline(resid(lme_SLAm_g_p_lan))


## Pseudo R^2 -lme
rsquared(lme_SLAm_g_p_lan)

##----------#
## LNC mean #
##----------#

#check normal distribution:
```

```
x11()
qqnorm(log(niche_p_sc$LNC)[niche_p_sc$species=="G"])
qqline(log(niche_p_sc$LNC)[niche_p_sc$species=="G"])
shapiro.test(log(niche_p_sc$LNC)[niche_p_sc$species=="G"])

### linear mixed effect model
## Interaction-Model with landolt values
lme_LNCm_g_p_lan<-lme(log(LNC) ~
cover_total*Richness+altitude+Eastness+lan_TEMP+lan_REACT+lan_NUTRI+lan_MOIST, random=
~1|glacier/transect/plot, data= niche_p_sc, subset=species=="G")
summary(lme_LNCm_g_p_lan)

#check assumtions:
x11()
qqnorm(resid(lme_LNCm_g_p_lan))
qqline(resid(lme_LNCm_g_p_lan))

## Pseudo R^2 -lme
rsquared(lme_LNCm_g_p_lan)

#-------------#
# Oxyria only #
#-------------#
##----------#
## SLA mean #
##----------#

## Interaction-Model with landolt values
lme_SLAm_o_p_lan<-lme(log(SLA_mean_plot) ~
cover_total*Richness+altitude+Eastness+lan_TEMP+lan_REACT+lan_NUTRI+lan_MOIST, random=
~1|glacier/transect/plot, data= niche_p_sc, subset=species=="O")
summary(lme_SLAm_o_p_lan)

#check assumtions:
x11()
qqnorm(resid(lme_SLAm_o_p_lan))
qqline(resid(lme_SLAm_o_p_lan))

## Pseudo R^2 -lme
rsquared(lme_SLAm_o_p_lan)

##----------#
## LNC mean #
##----------#

### linear mixed effect model
## Interaction-Model with landolt values
lme_LNCm_o_p_lan<-lme(log(LNC) ~
cover_total*Richness+altitude+Eastness+lan_TEMP+lan_REACT+lan_NUTRI+lan_MOIST, random=
~1|glacier/transect/plot, data= niche_p_sc, subset=species=="O")
summary(lme_LNCm_o_p_lan)

#check assumtions:
x11()
qqnorm(resid(lme_LNCm_o_p_lan))
qqline(resid(lme_LNCm_o_p_lan))

## Pseudo R^2 -lme
rsquared(lme_LNCm_o_p_lan)



#-----------------#
# Persicaria only #
#-----------------#
##----------#
## SLA mean #
##----------#

### linear mixed effect model
```

```
## Interaction-Model with landolt values
lme_SLAm_p_p_lan<-lme(log(SLA_mean_plot) ~
cover_total*Richness+altitude+Eastness+lan_TEMP+lan_REACT+lan_NUTRI+lan_MOIST, random=
~1|glacier/transect/plot, data= niche_p_sc, subset=species=="P")
summary(lme_SLAm_p_p_lan)

#check assumtions:
x11()
qqnorm(resid(lme_SLAm_p_p_lan))
qqline(resid(lme_SLAm_p_p_lan))

## Pseudo R^2 -lme
rsquared(lme_SLAm_p_p_lan)

##----------#
## LNC mean #
##----------#

### linear mixed effect model
## Interaction-Model with landolt values
lme_LNCm_p_p_lan<-lme(log(LNC) ~
cover_total*Richness+altitude+Eastness+lan_TEMP+lan_REACT+lan_NUTRI+lan_MOIST, random=
~1|glacier/transect/plot, data= niche_p_sc, subset=species=="P")
summary(lme_LNCm_p_p_lan)

#check assumtions:
x11()
qqnorm(resid(lme_LNCm_p_p_lan))
qqline(resid(lme_LNCm_p_p_lan))

## Pseudo R^2 -lme
rsquared(lme_LNCm_p_p_lan)

#================================================#

############################
### ecological gradients: ###
############################
#-------------#
# temperature #
#-------------#

## all species
gradient_temp<-lme(lan_TEMP~transect,random= ~1|species/glacier, data=niche_all)
summary(gradient_temp)
# transect: p=0

#check assumtions:
x11()
qqnorm(resid(gradient_temp))
qqline(resid(gradient_temp))

newdat_lme_temp = data.frame(transect = niche_all$transect, data=niche_all)
newdat_lme_temp$predlme = predict(gradient_temp, newdata = newdat_lme_temp, level = 0)

## all species
x11()
ggplot(data = niche_all,aes(x=factor(transect), y=lan_TEMP, fill=factor(glacier))) +
  geom_boxplot(position=position_dodge(1), show.legend = T)+ #remove legend
  scale_fill_manual(values=c("white", "grey80", "grey40"), name="glaciers",
labels=c("Frosnitzvalley", "Untersulzbachvalley", "Viltragenvalley"))+
  scale_y_continuous(limits=c(1,5), breaks =c(1,2,3,4,5))+
  theme_gray(base_size = 17) +
  xlab("transects") +
  ylab("temperatur index after landolt")+
  geom_line(data = newdat_lme_temp, aes(x=niche_all$transect, y = predlme,
group=factor(data.glacier),fill=NULL ))+
  annotate("text", x = 1.5, y = 4.75, label = "  p < 0.001***",size=6 )
# difference between glaciers
```

```
#check assumptions for Comparison between gla cier
x11()
qqnorm(niche_all$lan_TEMP)
qqline(niche_all$lan_TEMP)
shapiro.test(niche_all$lan_TEMP) #non normal -->Kruskal-Wallis Test

res.kruskal<-kruskal.test(lan_TEMP~glacier, data=niche_all)
pwc<-pairwise.wilcox.test(niche_all$lan_TEMP,niche_all$glacier, p.adjust.method =
"bonferroni")

my_comparisons <- list( c("1", "2"), c("2", "3"), c("1", "3") )
pwc_temp<-compare_means(lan_TEMP~glacier,data= niche_all,comparison=my_comparisons,
method = "wilcox.test",p.adjust.method = "bonferroni")

# custom graph because p.adj /= p.signif
df1 <- data.frame(a = c(1, 1:3,3), b = c(3, 3.1, 3.1, 3.1,3))
df2 <- data.frame(a = c(1, 1,2, 2), b = c(2.4, 2.5, 2.5, 2.4))
df3 <- data.frame(a = c(2, 2, 3, 3), b = c(2.7, 2.8, 2.8, 2.7))

# boxplot
x11()
ggplot(data = niche_all,aes(x=factor(glacier), y=lan_TEMP)) +
  geom_boxplot(position=position_dodge(1), show.legend = F,
aes(fill=factor(glacier)))+
  scale_fill_manual(values=c("white", "grey80", "grey40"), name="glaciers",
labels=c("Frosnitzvalley", "Untersulzbachvalley", "Viltragenvalley"))+
  scale_y_continuous(limits=c(1,5), breaks =c(1,2,3,4,5))+
  scale_x_discrete(labels=c("","",""))+
  theme_gray(base_size = 17) +
  xlab("glaciers") +
  ylab("temperatur index after landolt")+
  theme(plot.title = element_text(size=14))+
  geom_line(data = df1, aes(x = a, y = b)) + annotate("text", x = 2, y = 3.2, label =
"***", size = 6) +
  geom_line(data = df2, aes(x = a, y = b)) + annotate("text", x = 1.5, y = 2.6, label
= "***", size = 6) +
  geom_line(data = df3, aes(x = a, y = b)) + annotate("text", x = 2.5, y = 2.9 , label
= "**", size =6)+
  annotate("text", x = 1, y = 4.75, label = "  p < 0.001***",size=6 )

# geom_signif(comparisons = list( c("1", "2"), c("2", "3"), c("1",
"3")),map_signif_level=TRUE, y_position = c(2.5, 2.75, 3))# Option 1 ggsignif package
# stat_compare_means()+
# stat_compare_means(comparisons = my_comparisons, label.y = c(2.5, 2.75, 3),label =
"p.signif") #Option 2 ggpubr packages


#-----------#
# nutrients #
#-----------#

## all species
gradient_nutri<-lme(lan_NUTRI~transect,random= ~1|species/glacier, data=niche_all)
summary(gradient_nutri)


newdat_lme_nutri = data.frame(transect = niche_all$transect, data=niche_all)
newdat_lme_nutri$predlme = predict(gradient_nutri, newdata = newdat_lme_nutri, level =
0)

## all species
x11()
ggplot(data = niche_all,aes(x=factor(transect), y=lan_NUTRI, fill=factor(glacier))) +
  geom_boxplot(position=position_dodge(1), show.legend = F)+ #remove legend
  scale_fill_manual(values=c("white", "grey80", "grey40"), name="glaciers",
labels=c("Frosnitzvalley", "Untersulzbachvalley", "Viltragenvalley"))+
  scale_y_continuous(limits=c(1,5), breaks =c(1,2,3,4,5))+
  theme_gray(base_size = 17) +
  xlab("transects") +
  ylab("nutrient supply index after landolt")+
```

```
  geom_line(data = newdat_lme_nutri, aes(x=niche_all$transect, y = predlme,
group=factor(data.glacier),fill=NULL ))+
  annotate("text", x = 1.5, y = 4.75, label = " p < 0.001***",size=6 )

#check assumptions for Comparison between glacier
x11()
qqnorm(niche_all$lan_NUTRI)
qqline(niche_all$lan_NUTRI)
shapiro.test(niche_all$lan_NUTRI) #non normal -->Kruskal-Wallis Test
kruskal.test(lan_NUTRI~glacier, data=niche_all)
pairwise.wilcox.test(niche_all$lan_NUTRI,niche_all$glacier, p.adjust.method =
"bonferroni")
pwc_nutri<-compare_means(lan_NUTRI~glacier,data= niche_all,comparison=my_comparisons,
method = "wilcox.test",p.adjust.method = "bonferroni")

# custom graph because p.adj /= p.signif
df1 <- data.frame(a = c(1, 1:3,3), b = c(4.2, 4.3, 4.3, 4.3, 4.2))
df2 <- data.frame(a = c(1, 1,2, 2), b = c(3.6, 3.7, 3.7, 3.6))
df3 <- data.frame(a = c(2, 2, 3, 3), b = c(3.9, 4, 4, 3.9))

# boxplot
x11()
ggplot(data = niche_all,aes(x=factor(glacier), y=lan_NUTRI)) +
  geom_boxplot(position=position_dodge(1), show.legend = F,
aes(fill=factor(glacier)))+
  scale_fill_manual(values=c("white", "grey80", "grey40"), name="glaciers",
labels=c("Frosnitzvalley", "Untersulzbachvalley", "Viltragenvalley"))+
  scale_y_continuous(limits=c(1,5), breaks =c(1,2,3,4,5))+
  scale_x_discrete(labels=c("","",""))+
  theme_gray(base_size = 17) +
  xlab("glaciers") +
  ylab("nutrient supply index after landolt")+
  geom_line(data = df1, aes(x = a, y = b)) + annotate("text", x = 2, y = 4.4, label =
"***", size = 6) +
  geom_line(data = df2, aes(x = a, y = b)) + annotate("text", x = 1.5, y = 3.8, label
= "***", size = 6) +
  geom_line(data = df3, aes(x = a, y = b)) + annotate("text", x = 2.5, y = 4.1 , label
= "**", size =6)+
  annotate("text", x = 1, y = 4.75, label = "  p < 0.001***",size=6 )

#-------------#
# total cover #
#-------------#

## all species
gradient_cover<-lme(cover_total~transect,random= ~1|species/glacier, data=niche_all)
summary(gradient_cover)

newdat_lme_cover = data.frame(transect = niche_all$transect, data=niche_all)
newdat_lme_cover$predlme = predict(gradient_cover, newdata = newdat_lme_cover, level =
0)


## all species
x11()
ggplot(data = niche_all,aes(x=factor(transect), y=cover_total, fill=factor(glacier)))
+
  geom_boxplot(position=position_dodge(1), show.legend = FALSE)+
  scale_fill_manual(values=c("white", "grey80", "grey40"), name="glaciers",
labels=c("Frosnitzvalley", "Untersulzbachvalley", "Viltragenvalley"))+
  scale_y_continuous(limits=c(0,100), breaks =c(0,10,20,30,40,50,60,70,80,90,100))+
  theme_gray(base_size = 17) +
  xlab("transects") +
  ylab("Plant cover [%]")+
  geom_line(data = newdat_lme_cover, aes(x=niche_all$transect, y = predlme,
group=factor(data.glacier),fill=NULL ))+
  annotate("text", x = 1.5, y = 4.75, label = " p < 0.001***",size=6 )

#check assumptions for Comparison between glacier
x11()
```

```
qqnorm(niche_all$cover_total)
qqline(niche_all$cover_total)
shapiro.test(niche_all$cover_total) #non normal -->Kruskal-Wallis Test
kruskal.test(cover_total~glacier, data=niche_all)
pairwise.wilcox.test(niche_all$cover_total,niche_all$glacier, p.adjust.method =
"bonferroni")
pwc_cover<-compare_means(cover_total~glacier,data=
niche_all,comparison=my_comparisons, method = "wilcox.test",p.adjust.method =
"bonferroni")

df1 <- data.frame(a = c(1, 1:3,3), b = c(133, 138, 138, 138, 133))
df2 <- data.frame(a = c(1, 1,2, 2), b = c(113, 118, 118, 113))
df3 <- data.frame(a = c(2, 2, 3, 3), b = c(123, 128, 128, 123))

# boxplot
x11()
ggplot(data = niche_all,aes(x=factor(glacier), y=cover_total)) +
  geom_boxplot(position=position_dodge(1), show.legend = F,
aes(fill=factor(glacier)))+
  scale_fill_manual(values=c("white", "grey80", "grey40"), name="glaciers",
labels=c("Frosnitzvalley", "Untersulzbachvalley", "Viltragenvalley"))+
  scale_y_continuous(limits=c(0,150), breaks =c(0,10,20,30,40,50,60,70,80,90,100))+
  scale_x_discrete(labels=c("","",""))+
  theme_gray(base_size = 17) +
  xlab("glaciers") +
  ylab("Plant cover [%]")+
  geom_line(data = df1, aes(x = a, y = b)) + annotate("text", x = 2, y = 140, label =
"***", size = 6) +
  geom_line(data = df2, aes(x = a, y = b)) + annotate("text", x = 1.5, y = 120, label
= "***", size = 6) +
  geom_line(data = df3, aes(x = a, y = b)) + annotate("text", x = 2.5, y = 130 , label
= "**", size =6)+
  annotate("text", x = 1, y = 148, label = "  p < 0.001***",size=6 )

#----------#
# Richness #
#----------#

## all species
gradient_Richness<-lme(Richness~transect,random= ~1|species/glacier, data=niche_all)
summary(gradient_Richness)
# transect: p=2e-04

newdat_lme_Richness = data.frame(transect = niche_all$transect, data=niche_all)
newdat_lme_Richness$predlme = predict(gradient_Richness, newdata =
newdat_lme_Richness, level = 0)



## all species
x11()
ggplot(data = niche_all,aes(x=factor(transect), y=Richness, fill=factor(glacier))) +
  geom_boxplot(position=position_dodge(1), show.legend = FALSE)+
  scale_fill_manual(values=c("white", "grey80", "grey40"), name="glaciers",
labels=c("Frosnitzvalley", "Untersulzbachvalley", "Viltragenvalley"))+
  scale_y_continuous(limits=c(0,30), breaks =c(0,5,10,15,20,25,30))+
  theme_gray(base_size = 17) +
  xlab("transects") +
  ylab("Species Richness")+
  geom_line(data = newdat_lme_Richness, aes(x=niche_all$transect, y = predlme,
group=factor(data.glacier),fill=NULL ))+
  annotate("text", x = 1.5, y = 29, label = " p < 0.001***",size=6 )

  #check assumptions for Comparison between glacier
x11()
qqnorm(niche_all$Richness)
qqline(niche_all$Richness)
shapiro.test(niche_all$Richness) #non normal -->Kruskal-Wallis Test
kruskal.test(Richness~glacier, data=niche_all)
```

```
pairwise.wilcox.test(niche_all$Richness,niche_all$glacier, p.adjust.method =
"bonferroni")
pwc_Richness<-compare_means(Richness~glacier,data=
niche_all,comparison=my_comparisons, method = "wilcox.test",p.adjust.method =
"bonferroni")

# custom graph because p.adj /= p.signif
df1 <- data.frame(a = c(1, 1:3,3), b = c(34, 35,35, 35 ,34))
df2 <- data.frame(a = c(1, 1,2, 2), b = c(29, 30, 30 ,29))
df3 <- data.frame(a = c(2, 2, 3, 3), b = c(31, 32, 32,31))

x11()
ggplot(data = niche_all,aes(x=factor(glacier), y=Richness)) +
  geom_boxplot(position=position_dodge(1), show.legend = F,
aes(fill=factor(glacier)))+
  scale_fill_manual(values=c("white", "grey80", "grey40"), name="glaciers",
labels=c("Frosnitzvalley", "Untersulzbachvalley", "Viltragenvalley"))+
  scale_y_continuous(limits=c(0,40), breaks =c(0,5,10,15,20,25,30,35,40))+
  scale_x_discrete(labels=c("","",""))+
  theme_gray(base_size = 17) +
  xlab("glaciers") +
  ylab("Species Richness")+
  geom_line(data = df1, aes(x = a, y = b)) + annotate("text", x = 2, y = 37, label =
".", size = 8) +
  geom_line(data = df2, aes(x = a, y = b)) + annotate("text", x = 1.5, y = 31, label =
"**", size = 5) +
  geom_line(data = df3, aes(x = a, y = b)) + annotate("text", x = 2.5, y = 33.5 ,
label = "p > 0.1", size =4.5)+
  annotate("text", x = 1, y = 39, label = " p = 0.0027**",size=6 )

#----#
# pH #
#----#

## all species
gradient_react<-lme(lan_REACT~transect,random= ~1|species/glacier, data=niche_all)
summary(gradient_react)
# transect: p=0.1105

newdat_lme_react = data.frame(transect = niche_all$transect, data=niche_all)
newdat_lme_react$predlme = predict(gradient_react, newdata = newdat_lme_react, level =
0)

## all species
x11()
ggplot(data = niche_all,aes(x=factor(transect), y=lan_REACT, fill=factor(glacier))) +
  geom_boxplot(position=position_dodge(1), show.legend = F)+ #remove legend
  scale_fill_manual(values=c("white", "grey80", "grey40"), name="glaciers",
labels=c("Frosnitzvalley", "Untersulzbachvalley", "Viltragenvalley"))+
  scale_y_continuous(limits=c(1,5), breaks =c(1,2,3,4,5))+
  theme_gray(base_size = 17) +
  xlab("transects") +
  ylab("pH index after landolt")+
  geom_line(data = newdat_lme_react, aes(x=niche_all$transect, y = predlme,
group=factor(data.glacier),fill=NULL ))+
  annotate("text", x = 1.5, y = 4.75, label = " p = 0.111",size=6 )

#check assumptions for Comparison between glacier
x11()
qqnorm(niche_all$lan_REACT)
qqline(niche_all$lan_REACT)
shapiro.test(niche_all$lan_REACT) #non normal -->Kruskal-Wallis Test
kruskal.test(lan_REACT~glacier, data=niche_all)
pairwise.wilcox.test(niche_all$lan_REACT,niche_all$glacier, p.adjust.method =
"bonferroni")
pwc_pH<-compare_means(lan_REACT~glacier,data= niche_all,comparison=my_comparisons,
method = "wilcox.test",p.adjust.method = "bonferroni")

# custom graph because p.adj /= p.signif
df1 <- data.frame(a = c(1, 1:3,3), b = c(4.5, 4.6, 4.6, 4.6, 4.5))
```

```
df2 <- data.frame(a = c(1, 1,2, 2), b = c(4, 4.1, 4.1, 4))
df3 <- data.frame(a = c(2, 2, 3, 3), b = c(4.2, 4.3, 4.3, 4.2))

# boxplot
x11()
ggplot(data = niche_all,aes(x=factor(glacier), y=lan_REACT)) +
  geom_boxplot(position=position_dodge(1), show.legend = F,
aes(fill=factor(glacier)))+
  scale_fill_manual(values=c("white", "grey80", "grey40"), name="glaciers",
labels=c("Frosnitzvalley", "Untersulzbachvalley", "Viltragenvalley"))+
  scale_y_continuous(limits=c(1,5), breaks =c(1,2,3,4,5))+
  scale_x_discrete(labels=c("","",""))+
  theme_gray(base_size = 17) +
  ylab("temperatur index after landolt")+
  xlab("glaciers") +
  ylab("pH index after landolt")+
  geom_line(data = df1, aes(x = a, y = b)) + annotate("text", x = 2, y = 4.7, label =
"***", size = 6) +
  geom_line(data = df2, aes(x = a, y = b)) + annotate("text", x = 1.5, y = 4.2, label
= "***", size = 6) +
  geom_line(data = df3, aes(x = a, y = b)) + annotate("text", x = 2.5, y = 4.4 , label
= "**", size =6)+
  annotate("text", x = 1, y = 4.9, label = " p < 0.001***",size=6 )

#----------#
# moisture #
#----------#

## all species
gradient_moist<-lme(lan_MOIST~transect,random= ~1|species/glacier, data=niche_all)
summary(gradient_moist)
# transect: p=0

newdat_lme_moist = data.frame(transect = niche_all$transect, data=niche_all)
newdat_lme_moist$predlme = predict(gradient_moist, newdata = newdat_lme_moist, level =
0)

## all species
x11()
ggplot(data = niche_all,aes(x=factor(transect), y=lan_MOIST, fill=factor(glacier))) +
  geom_boxplot(position=position_dodge(1), show.legend = FALSE)+ #remove legend
  scale_fill_manual(values=c("white", "grey80", "grey40"), name="glaciers",
labels=c("Frosnitzvalley", "Untersulzbachvalley", "Viltragenvalley"))+
  scale_y_continuous(limits=c(1,5), breaks =c(1,2,3,4,5))+
  theme_gray(base_size = 17) +
  xlab("transects") +
  ylab("moisture index after landolt")+
  geom_line(data = newdat_lme_moist, aes(x=niche_all$transect, y = predlme,
group=factor(data.glacier),fill=NULL ))+
  annotate("text", x = 1.5, y = 4.75, label = " p < 0.001***",size=6 )

#check assumptions for Comparison between glacier
x11()
qqnorm(niche_all$lan_MOIST)
qqline(niche_all$lan_MOIST)
shapiro.test(niche_all$lan_MOIST) #non normal -->Kruskal-Wallis Test
kruskal.test(lan_MOIST~glacier, data=niche_all)
pairwise.wilcox.test(niche_all$lan_MOIST,niche_all$glacier, p.adjust.method =
"bonferroni")
pwc_moist<-compare_means(lan_MOIST~glacier,data= niche_all,comparison=my_comparisons,
method = "wilcox.test",p.adjust.method = "bonferroni")

# custom graph because p.adj /= p.signif
df1 <- data.frame(a = c(1, 1:3,3), b = c(4.4, 4.5, 4.5,4.5,4.4))
df2 <- data.frame(a = c(1, 1,2, 2), b = c(4.15, 4.25, 4.25, 4.15))
df3 <- data.frame(a = c(2, 2, 3, 3), b = c(3.9, 4, 4, 3.9))

# boxplot
x11()
ggplot(data = niche_all,aes(x=factor(glacier), y=lan_MOIST)) +
```

```
  geom_boxplot(position=position_dodge(1), show.legend = F,
aes(fill=factor(glacier)))+
  scale_fill_manual(values=c("white", "grey80", "grey40"), name="glaciers",
labels=c("Frosnitzvalley", "Untersulzbachvalley", "Viltragenvalley"))+
  scale_y_continuous(limits=c(1,5), breaks =c(1,2,3,4,5))+
  scale_x_discrete(labels=c("","",""))+
  theme_gray(base_size = 17) +
  xlab("glaciers") +
  ylab("moisture index after landolt")+
  geom_line(data = df1, aes(x = a, y = b)) + annotate("text", x = 2, y = 4.7, label =
"***", size = 4) +
  geom_line(data = df2, aes(x = a, y = b)) + annotate("text", x = 1.5, y = 4.35, label
= "p > 0.1", size = 4.5) +
  geom_line(data = df3, aes(x = a, y = b)) + annotate("text", x = 2.5, y = 4.1, label
= "***", size =4)+
  annotate("text", x = 1, y = 4.9, label = " p < 0.001***",size=6 )
#================================================#
```