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Elevational patterns and drivers of phyllosphere microbial diversity

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Elevational patterns and drivers of phyllosphere microbial diversity

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2
3 **17 Abstract:**
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5 **18 Aim:** Phyllosphere microbial diversity (PMD) is a central biodiversity component for plant health,
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8 **19** distribution, and ecosystem functioning. Yet, how climate and leaf functional traits of host plants
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10 **20** simultaneously shape elevational patterns of PMD is unclear; but it could improve our ability to
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12 **21** predict plant performance and distributions and ecosystem functioning under environmental
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15 **22** changes.
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17 **23 Location:** Temperate forests of Changbai Mountain Natural Reserve, China
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19 **24 Methods:** We investigated changes in the species richness and Shannon's diversity of endo- and
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22 **25** epiphytic phyllosphere fungal and bacterial communities, and their covariations, along an 1150 m
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24 **26** elevational gradient. We also examined the direct and indirect effects of climate (mean annual
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26 **27** temperature; MAT) and thirteen leaf morphological and chemical traits on PMD patterns.
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28 **28 Results:** We observed a monotonous decline in PMD with increasing elevation, contrasting with
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31 **29** the hump-shaped biodiversity patterns that are commonly reported. Host plant traits – those
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33 **30** involved in resource uptake and leaf surface temperature – predominantly shaped the elevational
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35 **31** patterns of endophytic PMD, whereas MAT mostly increased the richness and Shannon's diversity
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37 **32** of epiphytic organisms. We also **observed weak, but significant indirect effects** (up to 10%),
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39 **33** highlighting that host plant traits are important biotic drivers mediating climate effects on
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41 **34** endophytic PMD. Also, no covariation between bacteria and fungi was observed (neither for
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43 **35** endophytic nor for epiphytic organisms), supporting neutral associations between bacterial and
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45 **36** fungal communities, irrespective of the elevation.
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48
49 **37 Main conclusions:** Our results suggest considering both direct and mediating effects of plant traits
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51 **38** to better understand the drivers shaping the richness and Shannon's diversity of endo- and
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53 **39** epiphytic phyllosphere microbiomes, and more generally the plant-microbe associations. Our
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3 40 study also offers a trait-based attempt to disentangle the effects of biotic and abiotic filters in
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5 41 shaping endo- and epiphytic phyllosphere microbial diversity along an elevational gradient.
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8 42 **Keywords:** Biodiversity, Climate, Elevation, Plant functional traits, Plant-microbe associations,
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10 43 Microbial ecology.
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For Peer Review

45 Introduction

46 The phyllosphere – the aboveground part of plants – constitutes the largest surface area that
47 microbes inhabit on Earth, and is being estimated to be twice of the global land area (Vorholt,
48 2012; Zhu et al., 2021). The phyllosphere homes a large variety of phyllosphere microbes,
49 including endophytic and epiphytic fungi and bacteria (Phyllosphere Microbial Diversity;
50 hereafter PMD) (Vorholt, 2012). PMD is a central biodiversity component, largely involved in
51 plant health and performance, influencing ecosystem functioning and supporting crop production
52 (Vacher et al., 2016; Wang et al., 2021). Occurring at the interface between the leaf and the
53 atmosphere, PMD perceives the influences of abiotic (climate) and local biotic conditions (i.e. the
54 host plants). Investigating how PMD simultaneously responds to abiotic and biotic conditions
55 constitutes a great opportunity to better understand how the multiple environmental drivers shape
56 phyllosphere microbial communities, and may offer opportunities to better predict plant
57 performance and distribution, as well as ecosystem functioning under [global](#) environmental
58 changes.

59 Mountain ranges [harbor](#) many species endemic to high-elevation habitats, and exhibit multiple
60 but steep environmental changes at relatively small horizontal distances (Bryant et al., 2008;
61 Körner, 2000). For these reasons, elevational diversity gradients received considerable attention
62 (Rahbek et al., 2019). Organismal diversity could follow the early reported decreasing pattern with
63 higher elevation (Von Humboldt & Bonpland, 1805), following the notion that the environment
64 selects for a narrow and similar set of species (The environmental filtering hypothesis) (Le
65 Bagousse-Pinguet et al., 2017; Weiher, Clarke, & Keddy, 1998). However, a hump-shaped pattern
66 of diversity peaking at mid-elevations is likely to occur most commonly (Rahbek, 1995). These
67 diversity patterns have been documented for multiple taxa, such as soil microbes (Bryant et al.,

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3 68 2008), insects (Machac, Janda, Dunn, & Sanders, 2011; Sanders, 2002), birds (Duclos, DeLuca,
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5 69 & King, 2019), and plants (Grytnes & Beaman, 2006; Le Bagousse-Pinguet et al., 2018). However,
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8 70 we yet lack a clear understanding on how elevational - and underlying abiotic - gradients shape
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10 71 endo- and epiphytic PMD simultaneously. Determining whether PMD follow any particular
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12 72 pattern, and whether they follow the trends observed for other taxa may not only help to better
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15 73 understand the drivers shaping PMD patterns, but also extend, confirm, or refine the existing
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17 74 elevational diversity gradients to unknown taxa.

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19 75 Elevational diversity patterns have, yet, barely considered fine-scale biotic filters. For
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21 76 instance, the presence of cushion plants, either being competitive or offering micro-refuges, could
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24 77 mediate phylogenetic patterns of plant diversity along elevational gradients (Butterfield et al.,
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26 78 2013; Cavieres et al., 2014; Le Bagousse-Pinguet et al., 2018). Similarly, we could expect that
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29 79 host species act as an important biotic filter shaping, confounding or even altering the overall
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31 80 elevational patterns of PMD. The selection effect of plant host on phyllosphere microbes largely
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33 81 depends on plant attributes (i.e. plant functional traits) (Carvalho & Castillo, 2018; Jia, Yao, Guo,
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35 82 Wang, & Chai, 2020; Leveau, 2019; Vacher et al., 2016). Because plant functional traits relate to
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37
38 83 how species acquire and conserve resources (Díaz et al., 2007), they represent good proxies of the
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40 84 local biotic environment where phyllosphere microbial communities survive. In such a context,
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42 85 phyllosphere bacterial communities were tightly coupled with leaf dry matter content and leaf area,
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44 86 two plant functional traits involved in resource uptake (Kembel et al., 2014), while fungal
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47 87 communities mostly responded to leaf nitrogen content and specific leaf area (Kembel & Mueller,
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49 88 2014). However, host plants and their traits may exert greater impacts on the microbe colonization
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51 89 of internal tissues than on that of leaf surfaces, leading to potential distinct responses of epiphytic
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54 90 and endophytic microbes to the environment (Yao, Sun, He, Li, & Guo, 2020; Yao et al., 2019).

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3 91 For instance, endophytic fungi directly acquire nutrients from inner plant tissues, whereas
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5 92 epiphytic microbes in contact with the external environment mostly depend on the amount of
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7 93 nutrients either exuded from leaves or depositing from the atmosphere (Inácio et al., 2002). Hence,
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10 94 deciphering the relative effects of abiotic and biotic filters simultaneously shaping endo- and
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12 95 epiphytic PMD patterns remains unclear (Agler et al., 2016; Cordier et al., 2012; Wei et al., 2022),
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15 96 and constitutes an important step in our understanding of plant – microbe associations.

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17 97 Associations (covariations) between bacteria and fungi can arise from a diverse range of
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19 98 interactions, from negative to neutral or even positive. Negative associations could occur as a
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21 99 signature of competition for space and resources (Bahram et al. 2018), while niche partitioning
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23 100 may promote positive covariations as a result of fitness differences among organisms (Bruno,
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25 101 Stachowicz, & Bertness, 2003). For instance, fungal organic matter degradation has been shown
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27 102 to benefit bacteria (Boer, Folman, Summerbell, & Boddy, 2005). Yet, whether and how abiotic
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29 103 and biotic filters shape PMD associations remains poorly investigated (Agler et al., 2016; Cordier
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31 104 et al., 2012; Wei et al., 2022), even though describing how complex interactions among microbial
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33 105 communities could largely matter for the health of plants.

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37 106 Here, we investigated changes in the species richness and Shannon's diversity index of endo-
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39 107 and epi-phytic phyllosphere fungi and bacteria, and their covariations, along a steep 1150 m
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41 108 elevational gradient. We addressed the following questions and associated hypotheses: Q1) Does
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43 109 PMD exhibit particular elevational patterns? H1) We hypothesized that the diversity of
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45 110 phyllosphere microbes follows a mid-elevational gradient pattern, corresponding to the most
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47 111 common pattern observed across taxa. Q2) Do phyllosphere endo- and epiphytic microbes respond
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49 112 similarly to abiotic and biotic filters? H2) We hypothesized that endophytic microbes are more
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51 113 sensitive to the biotic filter (i.e., host plant traits), whereas epiphytic microbes mostly respond to
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3 114 climate. Q3) How do abiotic and biotic filters shape PMD covariations? H3) We hypothesized
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5 115 prominent negative relationships as a signature of competition for space and resources, and the
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8 116 elevational gradient to modulate these associations.
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11 118 **Material and Methods**

12 119 **Study site and climate data**

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17 120 The study site is located in the Changbai Mountain Natural Reserve (127° 42' to 128° 17' E,
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19 121 41° 43' to 42° 26' N) in the Jilin province of northeastern China (Fig. 1a), which is one of the
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22 122 largest protected temperate forests around the world (Stone, 2006; Wang et al., 2013). The reserve
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24 123 covers approximately 200, 000 ha, with an elevation range between 740 and 2691 m a.s.l. The area
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27 124 is strongly influenced by the monsoon and experiences a temperate continental mountain climate
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29 125 with long, cold winters and warm summers. The predominant soil type of the studied area is Alfisol
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31 126 according to the US soil taxonomy (Mao et al., 2019; Yang, 1985; Yuan et al., 2013).

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34 127 The elevational gradient considered in this study ranges from the mountain foot (500 m a.s.l.)
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36 128 to the summit (the volcanic crater lake) (2744 m a.s.l.) (Fig. 1b), where the mean annual
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38 129 temperature declines from 3.5 to -7.4 °C, but the mean annual precipitation (MAP) increases from
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40 130 632 to 1154 mm. The elevational gradient encompasses four distinct vegetation zones: the broad-
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43 131 leaved Korean pine (*Pinus koraiensis*) mixed forest (500 m–1100 m), the spruce-fir (*Picea*
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45 132 *jezoensis* and *Abies nephrolepis*) forest (1100–1800 m), the subalpine birch (*Betula ermanii*) forest
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47 133 (1800–2000 m) and an alpine tundra zone (above 2100 m). Along with a horizontal distance of
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50 134 about 40 km, these four forest types represent a condensed picture of the array of vegetation
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52 135 landscapes from the temperate zone to the polar region (Shao, Schall, & Weishampel, 1994; Yang,
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54 136 1985). [Data of mean annual temperature and precipitation were retrieved from \(Shen et al., 2014;](#)

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3 137 [Shen et al., 2013](#))

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8 139 **Leaf sampling**

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10 140 Leaf samples were collected in August 2020 at 800, 1000, 1200, 1400 1600, 1800 and 1950 m, i.e.

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12 141 at all representative forest zones along the studied elevational gradient (Table S1). [At each](#)

13
14 142 [elevation, we established five 50×50m plots that had an interval of 50 m and sampled 5 samples](#)

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16 143 [of each tree species from each elevation \(one sample per plot\) \(Fig1 c\). Because the number of](#)

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18 144 [dominant tree species differed among elevations \(there are 30, 20, 5,10, 20, 10 and 5 samples from](#)

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20 145 [the low to high elevation\), the total number of samples was not same among elevations \(Fig. 1b\).](#)

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22 146 In each plot, leaves without signs of disease or feeding damage were collected on one adult tree of

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24 147 each of the dominant species (Table S2). The specialized worker climbed upward the canopy of

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26 148 each tree by foot hook and took three branches from four directions using sterilized scissors. About

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28 149 80 gram of healthy leaves were taken using sterile gloves and then subdivided into two samples.

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30 150 One sample was stored at 4 °C to determine the leaf morphological and chemical properties,

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32 151 while the other sample was stored at -80 °C before DNA extraction. A total of 100 leaf samples

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34 152 were sampled from ten tree species (n=100, some species are distributed at multiple elevations).

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39 154 **Leaf functional traits**

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41 155 We measured 13 common leaf morphological and chemical traits related to life history and nutrient

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43 156 and water-use efficiencies (Table S3 & Fig. S1), following standard protocols (Pérez-

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45 157 Harguindeguy et al., 2013). Leaf morphological traits, such as specific leaf area (SLA), reflect

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47 158 light interception ability and trade-offs between the longevity of the plant tissues and construction

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49 159 cost, whereas leaf chemical traits such as leaf carbon (LCC) and leaf nitrogen content (LNC) reflect

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3 160 photosynthetic and growth ability (Chave et al., 2009). Leaf area (LA) was calculated using a
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5 161 portable scanner (Canon LiDE 110, Tokyo, Japan) and Image-Pro Plus 6.0 software (Media
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7 162 Cybernetics, USA). The mass of dried leaf samples (LDMC) with an accuracy of 0.1 mg was
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9 163 estimated after oven-drying at 65 °C for 48 hours at a constant mass. SLA was calculated as the
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11 164 ratio of projected leaf area to leaf dry mass. A RETSCH MM400 (RETSCH, GmbH, Haan,
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13 165 Germany) was then used to ground the oven-dried leaf samples into a fine powder. Leaf carbon
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15 166 and nitrogen contents were measured using an elemental analyzer (Vario EL III, Elementar, Hanau,
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17 167 Germany. Leaf stable carbon (LCC_{13}) and nitrogen isotope composition (LNC_{15}) was analyzed
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19 168 using the isotopic Cavity-Ring Down Spectrometer with the Combustion Module (CMCRDS
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21 169 system, Picarro, CA, USA). We also measured the following chemical elements: leaf phosphorus
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23 170 content (LPC), leaf potassium content (LKC), leaf copper content (LCuC), leaf calcium content
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25 171 (LCaC) and leaf zinc content (LZnC) using an ICP Optima 8000 (Perkin-Elmer, Waltham, MA,
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27 172 USA).

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29 173 We used the nail-polish imprint method (Zhao, Chen, Brodribb, & Cao, 2016) to estimate leaf
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31 174 stomatal character. Six leaves were randomly selected from each sample, and the nail polish was
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33 175 applied to the center of the abaxial leaf surface. After the nail polish is dried, the polish was then
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35 176 stripped using sellotape and mounted onto a glass slide. Microphotographs were photographed
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37 177 using a Classica SK200 Digital light microscope (Motic China Group Co., Ltd, China). 3-5 visual
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39 178 fields were randomly selected for each sample in a 40-fold objective lens and a 10-fold eye lens.
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41 179 Leaf stomatal area (LSA) was calculated as the mean value of the product of the length of the long
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43 180 axis and the length of the short axis of the five stomata selected in the random field using Image-
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45 181 Pro Plus 6.0 software (Media Cybernetics, USA).

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183 **Leaf DNA extraction and sequencing**

184 Phyllosphere epiphytic microbes were washed from leaf surfaces according to the standard
185 protocols (Yao et al., 2020; Yao et al., 2019). Five g of leaf samples were placed in sterile tubes
186 and 10 ml of sterile cooled TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) was added to each
187 gram of samples. Samples were then washed by ultrasonic for 1 min and whirled for 10 s, and
188 repeated twice. The liquids obtained after two cleanings were mixed and passed through a 0.2 µm
189 sterile filter membrane (SUPOR, Pall Corporation). The filtered membrane was transferred to the
190 freezer at -80 °C for analysis (Herrmann, Geesink, Richter, & Küsel, 2021). Samples of endophyte
191 microbes were isolated from the same plant leaves used for isolating epiphytes. The leaves were
192 submerged for 1 min with 75% ethanol, 3.25% sodium hypochlorite for 3 min, 75% ethanol for 30
193 s, and eventually rinsed 3 times with sterile distilled water. [Each sample was ground in liquid
194 nitrogen using a mortar and pestle, and then only 5 g of leaf powder was retained for DNA
195 extraction](#) (Bodenhausen, Horton, & Bergelson, 2013).

196 Total genomic DNA was extracted by the FastDNA® SPIN Kit (Qbiogene, Irvine, CA)
197 according to the manufacturer's instructions. The DNA extract was checked on 1% agarose gel,
198 and DNA concentration and purity were determined with NanoDrop 2000 UV-vis
199 spectrophotometer (Thermo Scientific, Wilmington, USA). For bacteria, we targeted V5-V7
200 region of the 16S rRNA gene, using 799F (AACMGGATTAGATACCCKG) and 1193R
201 (ACGTCATCCCCACCTTCC). [Two step PCR were used to amplify phyllosphere endophytic
202 bacteria. This method and the primer we choose can guarantee good coverage of endophytic
203 bacteria taxa](#) (Bulgarelli et al., 2015; Bulgarelli et al., 2012; Giangacomo, Mohseni, Kovar, &
204 [Wallace, 2021; Lundberg et al., 2012](#)). The PCR amplification was performed as follows: initial
205 denaturation step at 95°C for 3 min, the targeted region was amplified by 27 cycles of 95°C for 30

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3 206 s, 55°C for 30 s and 72°C for 45 s, followed by a final elongation step of 10 min at 72°C with the
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5 207 799F and 1392R (ACGGGCGGTGTGTRC) primers. With sterile deionized water, the initial PCR
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7 208 product was diluted by a factor of 50. 1.0 μ L of the diluted solution was used. The second-step
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9 209 primers were 799F-1193R and identical conditions to the first step except for the number of PCR
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11 210 cycles that was lowered to 13. For fungi, we targeted the ITS1 region of the rRNA operon, using
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13 211 the ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2R (GCTGCGTTCTTCATCGATGC).
14
15 212 The PCR amplification was performed as follows: initial denaturation at 95 °C for 3 min,
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17 213 followed by 35 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension
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19 214 at 72 °C for 45 s, and single extension at 72 °C for 10 min, and end at 10 °C. PCR amplification
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21 215 was performed for both the 16 S rRNA gene and the ITS1 region with 20 μ l reaction system,
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23 216 containing 5 \times TransStart FastPfu buffer 4 μ L, 2.5 mM dNTPs 2 μ L, forward primer (5 μ M) 0.8
24
25 217 μ L, reverse primer (5 μ M) 0.8 μ L, TransStart FastPfu DNA Polymerase 0.4 μ L, template DNA 10
26
27 218 ng, and finally ddH₂O up to 20 μ L. PCR reactions were performed in triplicate. The PCR product
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29 219 was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit
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31 220 (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and
32
33 221 quantified using Quantus™ Fluorometer (Promega, USA). The raw data were sequenced on an
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35 222 Illumina NovaSeq PE250 platform (Illumina, San Diego, USA) by Majorbio Bio-Pharm
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37 223 Technology Co. Ltd. (Shanghai, China).
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225 **Bioinformatic analyses**

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49 226 The raw gene sequencing reads were filtered using QIIME (version 1.7)
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51 227 (<http://qiime.org/tutorials/tutorial.html>). Low-quality sequences (length<200 bp), ambiguous bases>0,
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53 228 average base quality score<25) were removed. Samples were distinguished according to the
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barcode and primers, and the sequence direction was adjusted. The amplicon length ranges for 16S are 593bp (the first PCR) and 394bp (the second PCR), and the amplicon length ranges for ITS is 300bp (Yang et al., 2019). Only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of the overlap region is 0.2. Reads that could not be assembled were discarded; Samples were distinguished according to the barcode and primers, and the sequence direction was adjusted, exact barcode matching, 2 nucleotide mismatch in primer matching. The sequences were then clustered into operational taxonomic units (OTUs) with 97% similarity cutoff using UPARSE version 7.1 (Edgar, 2013), and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence of bacteria and fungi was assigned by RDP Classifier version 2.2 (Wang, Garrity, Tiedje, & Cole, 2007) against the Silva v138 with a confidence cut-off (*P*) value of 0.80 and UNITE 8.0 with a confidence cut-off (*P*) value of 0.70, respectively. The OTUs that were not classified into bacteria or fungi were removed. In total, we obtained 12,218,626 and 11,031,668 high-quality sequences for fungi and bacteria separately. After rarefying all samples to an equal sequencing depth (21898 reads per sample), the fungal and bacterial communities included 3110 and 5231 OTUs, respectively. The proportion of Chloroplasts (4/3173) and Mitochondria (30/3173) in the total number of OTUs was acceptable, and did not affect our analysis.

Statistical analyses

To evaluate the elevational patterns of PMD, we conducted linear mixed models with the Shannon index (*H*) (average value of each plot) and negative binomial generalized linear models with the richness (*S*) (average value of each plot) of all (i.e., epiphytic + endophytic), endo- and epiphytic bacteria and fungi as response variables, whereas elevation and elevation² (i.e. to test for potential

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3 252 humped-shaped relationships) as predictors, and the [plot as a random factor](#). We used negative
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5 253 binomial generalized linear models to account for the richness of microbes (e.g. the number of
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7 254 OTUs) in count data (Shrestha, Su, Xu, & Wang, 2018). Models were sorted and compared
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9 255 according to the Akaike Information Criterion (AIC) and the likelihood ratio test.
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12 256 [We performed a heatmap plot to illustrate the relationships between plant functional traits and](#)
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14 257 [specific microbial taxa by “corrplot” package \(Wei et al., 2017\)](#). We further performed a principal
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16 258 component analysis (PCA) on the nine least correlated leaf traits (over the 13 traits originally
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18 259 considered; Fig. S1). This approach approximates the functional space that species occupy and
19
20 260 defines their position in multi-dimensional trait space (Devictor et al., 2010). The first axis of PCA
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22 261 (FT_{PC1}) explained 46.9% of the total variation in trait variables. This axis mainly correlated with
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24 262 high leaf area and SLA (Table S4), thus discriminating leaf traits associated with the leaf economic
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26 263 spectrum (LES; Wright *et al.*, 2004). The second axis (FT_{PC2}) explained 28.7% of the total
27
28 264 variation in trait variables, and mainly correlated with micronutrients such as leaf copper and zinc
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30 265 contents. The two PCA axes were used in subsequent analyses because they are independent
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32 266 variables at the species level and reflect important leading dimensions of the species niche (Gross
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34 267 *et al.*, 2013).
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40 268 To evaluate the simultaneous effects of abiotic (climate) and biotic (leaf traits) conditions on
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42 269 endo- and epiphytic PMD, we performed linear mixed models with H and with S of all, endo- and
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44 270 epiphytic bacteria and fungi as response variables. We included MAT and MAT^2 as climate
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46 271 predictors (MAP was considered, but removed due to its high correlation with MAT and less
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48 272 importance in the models; Fig. S1), FT_{PC1} and FT_{PC2} as biotic predictors, and site included as a
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50 273 random factor. Response variables were log-transformed when necessary to normalize data
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52 274 distribution prior to analyses to meet the assumptions of the test used. All predictors were
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3 275 standardized using the Z-score to interpret parameter estimates on a comparable scale. Models
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5 276 were ranked according to the lowest AICc, and retained within $\Delta AIC < 2$. Standardized regression
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8 277 coefficients (β) were obtained using a model averaging approach. Since all predictors and response
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10 278 variables were standardized, an analogue of the variance decomposition analysis was applied to
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12 279 obtain the relative importance of each predictor based on the marginal R^2 , which can be simply
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14 280 calculated as the ratio between its standardized regression coefficient and the sum of all
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16 281 coefficients, and expressed in % (Le Bagousse-Pinguet et al., 2019; Yuan et al., 2021).

19 282 Finally, piecewise structural equation models (pSEMs) were performed to investigate direct
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21 283 and indirect pathways of the abiotic and biotic influences on PMD and their associations (the
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23 284 conceptual model is provided in Fig. S4). Each predictor's indirect impact was estimated by
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25 285 combining the standardized direct effect of a predictor on a mediator with the direct influence of
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27 286 a mediator on the response variable. The total effect of predictors is calculated as the sum of direct
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29 287 and indirect effects (Yuan et al., 2020). Based on a priori theoretical knowledge, we hypothesized
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31 288 that functional traits and climate factors simultaneously would have a direct impact on PMD (e.g.
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33 289 S of bacteria and fungi), and climate factors also would have an indirect impact on PMD via
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35 290 altering functional traits. We also considered potential relationships between bacteria and fungi to
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37 291 evaluate the strength and direction of their associations using **linear mixed models** in pSEM
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39 292 analyses. Site was used as a random factor. Model parameters and fit were assessed using Fisher's
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41 293 C statistic. Models with adequate fit to the data had a Fisher's C statistic with $p > 0.05$ (Shipley,
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43 294 2009). The pSEM analyses were performed using “piecewiseSEM” package (Lefcheck, 2016).
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45 295 The pSEMs were tested for S and H of phyllosphere epiphytic, endophytic and all microbial groups
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47 296 (epiphytic + endophytic).

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54 297 All statistical analyses were performed in the R 4.1.0 software (<http://www.r-project.org/>).

298 The negative binomial generalized linear models were conducted using the *MASS* package. The
299 linear mixed models were conducted using the *lme4* package, and model selection was performed
300 using the “dredge” function in the *MuMIn* package (Bartoń, 2020).

301
302 **Results**
303 We found that endo- and epiphytic bacteria were mainly composed of Proteobacteria and
304 Actinomycetes, while phyllosphere fungi were mainly represented by Ascomycetes and
305 Basidiomycetes (Appendix Fig. S2 & S3). Proteobacteria (the most dominant bacterial group)
306 were evenly distributed among the studied tree species, while the dominant groups of fungi
307 (Ascomycetes) were mainly associated to *Larix_gmelinii* and *Betula_ermanii*. The phyllosphere
308 microbes mostly occurred at low (800m) and mid-elevation (1600m).

309 For all studied response variables, the best—fitted models included elevation only as a
310 predictor (Fig.2; Appendix Tables S5 & S6). We observed a linear decrease pattern in richness (S)
311 and Shannon index (H) for both bacterial and fungal communities along the studied elevational
312 gradient. We observed a steeper decline in epiphytic bacterial diversity ($Bacteria_{EPIP}$) than in
313 endophytic bacteria ($Bacteria_{ENDO}$) (ANOVA test' $P < 0.001$, Fig.1), whereas endophytic fungi
314 ($Fungi_{ENDO}$) decreased more dramatically along the elevational gradient than epiphytic fungi
315 ($Fungi_{ENDO}$) (ANOVA test' $P < 0.001$).

316 The climate (MAT) and the multiple leaf traits accounted for a fair amount of explained
317 variance across the richness (S) of phyllosphere microbes (from 50 to 66%), and notably for
318 epiphytic fungi (Fig.3f). Mean annual temperature (MAT) explained on average 27% of variations
319 (19%–37%) in S of phyllosphere microbes. The climate (MAT) and the multiple leaf traits
320 explained a slightly higher amount of variance (from 56% to 69%) for the Shannon index (H), and

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2
3 321 notably for the epiphytic fungi (Fig.S5f). Mean annual temperature (MAT) explained up to 30%
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5 322 (22%–38%) of variations in H of phyllosphere microbes. MAT was significantly associated with
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7 323 higher S and H of microbial communities, indicating that higher temperature linearly increased
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9 324 both the richness and Shannon's diversity of microbes (MAT²: $p > 0.05$). The first axis of PCA on
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11 325 functional traits (FT_{PC1}) – related to the LES - explained up to 26% (13%–37%) and 28% (15%–
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13 326 41%) for S and H, respectively. Higher leaf area and SLA (and lower LDMC) thus increased the
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15 327 S and H of both bacterial and fungal communities. In contrast, the second axis of PCA on
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17 328 functional traits (FT_{PC2}) accounted for only 1.2% (1.1%–1.4%) of variations in PMD (Fig.3
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19 329 &Fig.5).

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24 330 The final pSEMs indicated that all PMD metrics were not only associated with direct
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26 331 changes in MAT and leaf traits but also through indirect pathways (Fig. 4; Appendix Table S7 &
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28 332 S8). Indirect effects represented up to 12% of variations in PMD. Phyllosphere bacterial S and H
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30 333 were thus directly influenced by MAT ($\beta=0.305-0.535$) and FT_{PC1} ($\beta=0.258-0.419$), and indirectly
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32 334 affected by trait-mediated effects of MAT ($\beta= 0.058-0.094$). The average total impact (all,
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34 335 endophytic and epiphytic) of MAT on phyllosphere bacterial S and H was 0.485 and 0.504,
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36 336 respectively. Phyllosphere fungal S and H were directly influenced by MAT ($\beta=0.498-0.603$) and
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38 337 FT_{PC1} ($\beta=0.333-0.508$), but indirectly affected by trait-mediated effects of MAT ($\beta= 0.074-0.114$).
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40 338 The average total impact (all, endophytic and epiphytic) of MAT on phyllosphere fungal S and H
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42 339 index were 0.515 and 0.544, respectively. Also, the effects of MAT and FT_{PC1} on phyllosphere
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44 340 fungi (0.388-0.603 and 0.333-0.508) were greater than those on phyllosphere bacteria (0.305-0.535
45
46 341 and 0.258-0.424). In sum, the effects of MAT on phyllosphere epiphytic microbes (0.524-0.603)
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48 342 were greater than those on phyllosphere endophytic microbes (0.305-0.571). The effects of FT_{PC1}
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50 343 on phyllosphere endophytic microbes (0.417-0.508) were greater than those on phyllosphere
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3 344 epiphytic microbes (0.258-0.359). Finally, we did not observe any correlations between bacteria
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5 345 and fungi, neither for endophytic nor epiphytic microbes ($P>0.05$). In addition, there was no strong
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7 346 change in the pSEMs results across S and H of phyllosphere epiphytic, endophytic and all
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9 347 microbial groups (Figs. 4, S6, S7 and S8).
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14 349 **Discussion**

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17 350 Here, we investigated the elevational patterns of endo- and epiphytic phyllosphere fungal and
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19 351 bacterial communities, and whether underlying climate (MAT) and biotic drivers (the leaf traits of
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21 352 host plants) could explain the observed patterns. We observed a linear decrease in the richness and
22
23 353 Shannon's diversity of fungal and bacterial communities along the studied elevational gradient,
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25 354 but contrary to expectation, no covariations between microbial communities were observed. The
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27 355 climate features and leaf plant functional traits shaped the observed monotonous decline of
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29 356 microbial biodiversity, with a higher sensitivity of endophytic microbes to the biotic filter (host
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31 357 plant traits) and epiphytic microbes to climate.
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35 358 We observed a monotonous decline of phyllosphere microbial diversity with increasing
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37 359 elevation, contrasting with the hump-shaped biodiversity pattern commonly reported across taxa
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39 360 (Rahbek, 1995), and notably for soil microbes (Geml et al., 2022; Kotilinek et al., 2017; Rehakova,
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41 361 Chlumska, & Dolezal, 2011). While restricting our study to areas above 700 m.a.s.l. (i.e. the areas
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43 362 that are the least impacted by human disturbance) may have limited our ability to detect humped-
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45 363 shaped patterns, our observed PMD patterns concurred with that of tropical plant species richness
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47 364 decreasing with elevation (Von Humboldt & Bonpland, 1805); and hence, with the view that the
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49 365 environmental filtering hypothesis (Le Bagousse-Pinguet et al., 2017; Weiher et al., 1998). Our
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51 366 result also matched with the clustering patterns observed for phylogenetic diversity with elevation
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3 367 for soil microbes (Bryant et al., 2008), insects (Machac et al., 2011; Smith, Hallwachs, & Janzen,
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5 368 2014), birds (Graham, Parra, Rahbek, & McGuire, 2009), and somehow for plants (Bryant et al.,
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8 369 2008; Zhang, Xiao, & Li, 2015). However, we also observed that the monotonous decline of PMD
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10 370 patterns along the elevational gradient was significantly steeper for epiphytic than endophytic
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12 371 bacteria; but the inverse was observed for fungal communities. These results highlight the complex
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14 372 responses of phyllosphere biological groups to environmental gradients, and the importance to
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16 373 discriminate endo- vs. epiphytic microbial communities if we aim at better understanding and
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18 374 predicting microbial changes in response to ongoing environmental changes. Altogether, our study
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20 375 extends our understanding of how elevation shapes the diversity of phyllosphere bacteria and fungi,
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22 376 and brings new evidence that hump-shaped biodiversity patterns may not be as prevalent as
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24 377 previously thought along elevational gradients (Guo et al., 2013; Wang et al., 2017).
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28 378 Our results reveal that temperature (and the highly collinear effect of the mean annual
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30 379 precipitation (MAP; Appendix Fig. S1)) was a key driver of PMD along the studied gradient, but
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32 380 it predominantly shaped the elevational patterns of epiphytic organisms. While MAT has been
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34 381 recognized as one of the main abiotic drivers of soil microbes along elevational gradients
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36 382 (Nottingham et al., 2018), here we showed that the climate impacts depend on how microbes are
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38 383 exposed to external conditions (Gomes, Pereira, Benhadi-Marín, Lino-Neto, & Baptista, 2018;
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40 384 Vacher, Cordier, & Vallance, 2016). The observed positive temperature-diversity relationship
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42 385 could be explained by the metabolic theory of ecology, i.e., temperature could accelerate the
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44 386 metabolic rates and biochemical processes of organisms (Brown, Gillooly, Allen, Savage, & West,
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46 387 2004; Gillooly, Brown, West, Savage, & Charnov, 2001). In addition, we observed that MAT has
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48 388 a slightly higher impact on the phyllosphere fungal than on bacterial diversity, concurring with the
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50 389 view that fungi are more sensitive to temperature changes than bacteria (Vacher et al., 2016).
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3 390 Altogether, our study warns for the potential changes that phyllosphere microbes could experience,
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5 391 and the potential for this central biodiversity component to further alter plant health, diversity and
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7 392 ecosystem functioning under global environmental changes such as warming.
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10 393 We found a significant effect of leaf functional traits on elevational patterns of PMD. Our
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12 394 result points toward the importance of the functional attributes of host plants for PMD, while we
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14 395 even did not consider plant phylogeny, a biotic driver that encompasses unmeasured biological
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16 396 traits (Flynn, Mirotchnick, Jain, Palmer, & Naeem, 2011) and is relevant for phyllosphere diversity
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18 397 (Leff et al., 2018; Martínez-García, Richardson, Tylianakis, Peltzer, & Dickie, 2015; Yang et al.,
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20 398 2019). Interestingly, we showed that leaf traits were mostly involved in shaping elevational
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22 399 patterns of endophytic PMD, likely because they predominantly depend on the inner plant tissues
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24 400 (Mina, Pereira, Lino-Neto, & Baptista, 2020; Yao et al., 2019). We further observed weak, but
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26 401 significant indirect effects (up to 10%), highlighting that host plant traits are important biotic
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28 402 drivers mediating climate effects on PMD. These indirect effects were 2-fold stronger for
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30 403 endophytic than epiphytic communities, reinforcing the primary role of host plant traits in shaping
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32 404 inner microbial communities. We acknowledge that our study did not consider interactive effects
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34 405 between MAT and the biotic attributes of host plants, and it may have potentially underestimated
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36 406 the strength of climate effects mediated by the local biotic filters. Our data also showed that the
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38 407 effects of host plant traits were slightly stronger on phyllosphere fungal diversity than on bacterial
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40 408 diversity. This result may arise from the higher transport material capacity of fungal mycelium
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42 409 than that of bacteria (Boer et al., 2005; Jennings, 1987). While studies on mountain diversity
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44 410 patterns yet barely considered fine-scale biotic filters, our results clearly highlight the need to
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46 411 consider their direct and mediating effects to better understand plant–microbe associations and the
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48 412 drivers shaping elevational diversity gradients.
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3 413 The first axis of the PCA was positively related to leaf traits such as leaf area and SLA (and
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5 414 negatively to LDMC; see Appendix Table S4). These leaf traits all belong to the Leaf Economic
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7 415 Spectrum (Wright et al., 2004), one of the major axes of the global spectrum of plant forms and
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9 416 functions (Diaz et al., 2016). They also represent influential plant functional attributes for
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11 417 phyllosphere bacterial (LDMC and LA) and fungal (SLA) diversities (Kembel & Mueller, 2014).
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14 418 Two main reasons could explain the importance of these functional attributes of host plants for
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16 419 phyllosphere microbial diversity. First, they discriminate fast-growing acquisitive strategies and
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18 420 resource uptake in plants (Kembel & Mueller, 2014), and thus relate to the plant's nutritional value
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20 421 (Leveau, 2019; Liu, Brettell, & Singh, 2020 ; Vorholt, 2012). Second, traits such as LDMC have
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22 422 been recently shown to relate to the leaf surface temperature (Liancourt, Song, Macek, Santrucek,
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24 423 & Dolezal, 2020; Michaletz et al., 2015; Yang et al., 2021), a biophysical process involved in plant
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26 424 species distribution along elevational gradients (Liancourt et al., 2020). As such, we may expect
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28 425 that LDMC could also determine the bioclimate that PMD experience, and likely their distributions
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30 426 along environmental gradients. While species-level trait measurements offer insights into PMD
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32 427 patterns, we further advocate for incorporating intraspecific trait variation, a central component
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34 428 influencing plant species interactions (Kraft, Crutsinger, Forrestel, & Emery, 2014; Le Bagousse-
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36 429 Pinguet et al., 2015), and community assembly and dynamics (Bolnick et al., 2011; Violle et al.,
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38 430 2012). Adopting an individual perspective of trait variation may further help to understand PMD
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40 431 patterns, as well as to refine our predictions for community assembly, functional ecology and
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42 432 plant–microbe associations.
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49 433 Finally, we did not observe any covariation between bacteria and fungi, neither for endophytic
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51 434 nor for epiphytic organisms, irrespective of the diversity metric considered (richness or Shannon's
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53 435 diversity). Our result supports neutral associations between bacterial and fungal communities,
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3 436 while we expected negative associations to occur as a signature of competition for space and
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5 437 resources (Bahram et al., 2018). Two reasons may explain these results. On the one hand, the
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7 438 absence of a relationship may highlight that stochastic assembly processes dominate as often
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9 439 observed in other hyperdiverse systems such as the tropical forest biome (Harms, Condit, Hubbell,
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11 440 & Foster, 2001). Neutral associations have been, for instance, observed in topsoil microbiomes,
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13 441 particularly in agricultural systems (Jiao et al., 2021). On the other hand, neutral associations could
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15 442 reflect the outcome of antagonistic deterministic processes fluctuating over time to shape microbial
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17 443 communities (Götzenberger et al., 2012; Wang et al., 2016).
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23 24 445 **Conclusions**

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26 446 Our results offer the first step in understanding PMD associations and call for further investigation
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28 447 to identify the putative underlying processing involved in shaping complex interactions among
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30 448 microbial communities. We found a monotonous decline of phyllosphere microbial diversity with
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32 449 increasing elevation, which contrasts with the commonly reported hump-shaped biodiversity
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34 450 pattern along elevational gradients. Our work also demonstrated that host plant functional traits –
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36 451 those involved in resource uptake and leaf surface temperature – predominantly shape endophytic
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38 452 PMD patterns through both direct and indirect effects, while climate mostly impacted epiphytic
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40 453 microbial communities. Our study provides a first hierarchical framework based on the simple
41
42 454 effect of traits to quantify the effects of biotic filters in shaping PMD patterns along ecological
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44 455 gradients, and to disentangle their effects from those of the abiotic environment.
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49 456 **Conflict of Interest Statement**

50
51 457 We ensure that none of the authors declared a conflict of interest.
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459 **Author contributions**

460 ZY, XW and YLB-P conceived the idea; XW, ZY, FL, ZM, JY, SF, ZH and XW performed the
461 research and collected the data; XW, AA, ZY and YLB-P analysed the data; XW and YLB-P wrote
462 the first draft of the manuscript, and all of the authors contributed substantially to the revisions.

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464 **Data Availability**

465 Microbe data are available from the NCBI Sequence Read Archive (SRA) database
466 (Accession Number: PRJNA824213), other data that support the findings of this study are
467 available from the corresponding author upon reasonable request.

468

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474 521100221033).

475

476 **Supporting Information**

477 **Fig. S1** Spearman correlations among candidate predictors (the abbreviations of leaf traits were
478 showed in Table S3)

479 **Fig. S2** Distribution of main phyla of phyllosphere microbes (the abbreviations of tree species
480 were shown in Table S1)

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3 481 **Fig. S3** Relative abundances of the dominant phylum and class for bacterial and fungal in the
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5 482 phyllosphere separated according to elevation categories. There were significant differences
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7 483 between communities at different elevations. Abbreviations: Bacteria_{All}, all bacteria;
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9 484 Bacteria_{Endo}, endophytic bacteria; Bacteria_{Epip}, epiphytic bacteria; Fungi_{All}, all fungi; Fungi_{Endo},
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11 485 endophytic fungi; Fungi_{epip}, epiphytic fungi.

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14 486 **Fig. S4** A conceptual model revealing the expected links of abiotic factors (mean annual
15
16 487 temperature) and biotic factors (leaf function traits) on phyllosphere microbial diversity.
17
18 488 Abbreviations: Bacteria_{All}, all bacteria; Bacteria_{Endo}, endophytic bacteria; Bacteria_{Epip}, epiphytic
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20 489 bacteria; Fungi_{All}, all fungi; Fungi_{Endo}, endophytic fungi; Fungi_{epip}, epiphytic fungi.

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22 490 **Fig. S5** Effects of mean annual temperature, plant identity(CWM_{MH}), and leaf functional traits
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24 491 on phyllosphere bacterial (a, c, d) and fungal (b, e, f) Shannon's diversity. We present the
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26 492 standardized regression coefficients of model predictors, and the associated 95% confidence
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28 493 intervals. We also present the relative importance of each predictor (expressed as the percentage
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30 494 of total variance). Significance levels are **: $P < 0.01$; ***: $P < 0.001$. Abbreviations: H,
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32 495 Shannon's diversity; MAT, mean annual temperature; MAT², the square of mean annual
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34 496 temperature; bacteria_{All}, all bacteria; Bacteria_{Endo}, endophytic bacteria; Bacteria_{Epip}, epiphytic
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36 497 bacteria; Fungi_{All}, all fungi; Fungi_{Endo}, endophytic fungi; Fungi_{epip}, epiphytic fungi; CWM_{MH}, the
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38 498 community-weighted mean of maximum tree height; FT_{PC1}, the first PCA axis on the nine
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40 499 functional traits considered; FT_{PC2}, the second PCA axis.

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45 500 **Figure. S6** Piecewise structural equation models (pSEMs) exploring the direct and indirect effects
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47 501 of mean annual temperature and leaf functional traits on phyllosphere bacterial and fungi
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49 502 Shannon's diversity. Standardized regression coefficients and significance are given (*<0.05,
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51 503 **<0.01). The effect sizes of direct and indirect paths are also presented. Abbreviation: H,
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3 504 Shannon's diversity; $Bacteria_{All}$, all bacteria; $Bacteria_{Endo}$, endophytic bacteria; $Bacteria_{Epip}$,
4 epiphytic bacteria; $Fungi_{All}$, all fungi; $Fungi_{Endo}$, endophytic fungi; $Fungi_{epip}$, epiphytic fungi; FT_{pc1} ,
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6 505 the first PCA axis on the nine functional traits studied; MAT, mean annual temperature.
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11 507 **Figure. S7** Piecewise structural equation models (pSEMs) exploring the effects of mean annual
12 temperature and leaf functional traits on phyllosphere bacterial and fungi richness. Standardized
13 regression coefficients and significance are given (* <0.05 , ** <0.01). Abbreviation: S, richness;
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15 508
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17 509
18 510 $Bacteria_{All}$, all bacteria; $Bacteria_{Endo}$, endophytic bacteria; $Bacteria_{Epip}$, epiphytic bacteria; $Fungi_{All}$,
19 all fungi; $Fungi_{Endo}$, endophytic fungi; $Fungi_{epip}$, epiphytic fungi; FT_{pc1} , the first PCA axis on the
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26 513 **Figure. S8** Piecewise structural equation models (pSEMs) exploring the effects of mean annual
27 temperature and leaf functional traits on phyllosphere bacterial and fungi Shannon's diversity.
28 Standardized regression coefficients and significance are given (* <0.05 , ** <0.01). Abbreviation:
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33 516 H, Shannon's diversity; $Bacteria_{All}$, all bacteria; $Bacteria_{Endo}$, endophytic bacteria; $Bacteria_{Epip}$,
34 epiphytic bacteria; $Fungi_{All}$, all fungi; $Fungi_{Endo}$, endophytic fungi; $Fungi_{epip}$, epiphytic fungi; FT_{pc1} ,
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41 519 **Figure. S9** Heatmap that shows relationship between plant functional traits and specific microbial
42 taxa (in the phyla level).
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48 522 **Table S1** Summary of the main characteristics of sampling sites along an elevational gradient on
49 Changbai Mountain.
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52 524 **Table S2** Geographic information of the studied sites.
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54 525 **Table S3** Functional traits and their functions.
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3 526 **Table S4** Loadings of the Principal Component Analysis for trait values.
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For Peer Review

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4 527 **Table S5** AIC value for predicting the relationship between phyllosphere microbial
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6 528 richness and elevation(elevation²). Bold represents the optimal model based on AIC.
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9 529 *: $P < 0.01$.

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11 530 **Table S6** AIC value for predicting the relationship between phyllosphere microbial
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14 531 Shannon index and elevation (elevation²). Bold represents the optimal model based on
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17 532 AIC. *: $P < 0.01$.

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19 533 **Table S7** The direct, indirect, and total standardized effects of mean annual
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22 534 temperature and leaf functional traits on phyllosphere bacterial and fungal richness
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25 535 derived from the pierce structural equation models. Models are presented in Fig. 4.
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27 536 Only significant effects are shown.

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29 537 **Table S8** The direct, indirect, and total standardized effects of mean annual
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32 538 temperature and leaf functional traits on phyllosphere bacterial and fungal Shannon
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35 539 index derived from the pierce structural equation models. Models are presented in Fig.
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37 540 S5. Only significant effects are shown.

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40 541 Figure legends

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43 542 **Figure 1.** The geographical position of the Changbai Mountain in north-east China (a),
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45 543 the location of study sites with the respective dominant forests (pictures) along an
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48 544 elevational gradient from 800 m to 1950 m above sea level (b), and the design of the
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51 545 study plot for samples collections (c).

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53 546 **Figure 2.** The richness of phyllosphere bacteria (a, b) and phyllosphere fungi (c, d)
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56 547 along the studied elevational gradient. Abbreviations: S, richness; H, Shannon index;

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4 548 Bacteria_{All}, all bacteria; Bacteria_{Endo}, endophytic bacteria; Bacteria_{Epip}, epiphytic
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6 549 bacteria; Fungi_{All}, all fungi; Fungi_{Endo}, endophytic fungi; Fungi_{epip}, epiphytic fungi.
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10 **Figure 3.** Effects of mean annual temperature, tree functional identity (CWM_{MH}), and
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12 leaf functional traits on phyllosphere bacterial (a, c, d) and fungal (b, e, f) richness. We
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14 present the standardized regression coefficients of model predictors and the associated
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16 552 95% confidence intervals. We also present the relative importance of each predictor
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18 553 (expressed as the percentage of total variance). Significance levels are **: $P < 0.01$;
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20 554 ***: $P < 0.001$. Abbreviations: S, richness; MAT, mean annual temperature; MAT²,
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22 the square of mean annual temperature; Bacteria_{All}, all bacteria; Bacteria_{Endo},
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24 555 endophytic bacteria; Bacteria_{Epip}, epiphytic bacteria; Fungi_{All}, all fungi; Fungi_{Endo},
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26 556 endophytic fungi; Fungi_{epip}, epiphytic fungi; FT_{PC1}, the first PCA axis on the nine
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28 557 functional traits considered; FT_{PC2}, the second PCA axis.
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561 **Figure 4.** Piecewise structural equation models (pSEMs) exploring the direct and
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563 indirect effects of mean annual temperature and leaf functional traits on phyllosphere
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565 bacterial and fungi richness. Standardized regression coefficients and significance are
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567 given ($* < 0.05$, $** < 0.01$). The effect sizes of direct and indirect paths are also presented.
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569 Abbreviation: S, richness; Bacteria_{All}, all bacteria; Bacteria_{Endo}, endophytic bacteria;
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571 Bacteria_{Epip}, epiphytic bacteria; Fungi_{All}, all fungi; Fungi_{Endo}, endophytic fungi;
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573 Fungi_{epip}, epiphytic fungi; FT_{pc1}, the first PCA axis on the nine functional traits studied;

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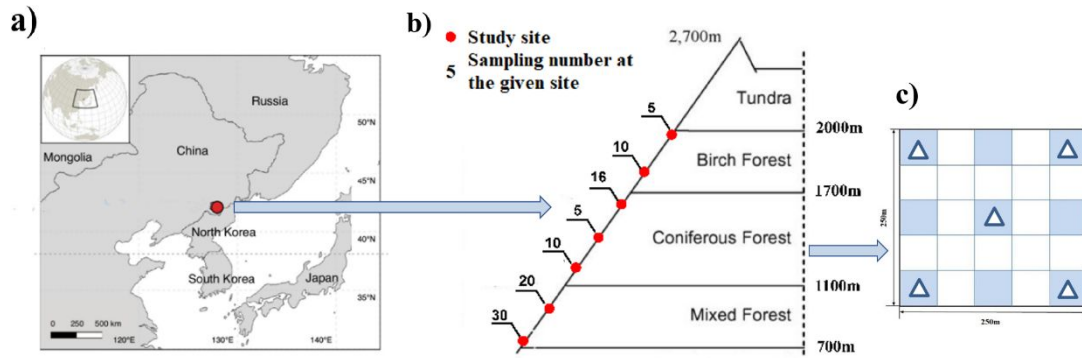


568 MAT, mean annual temperature.

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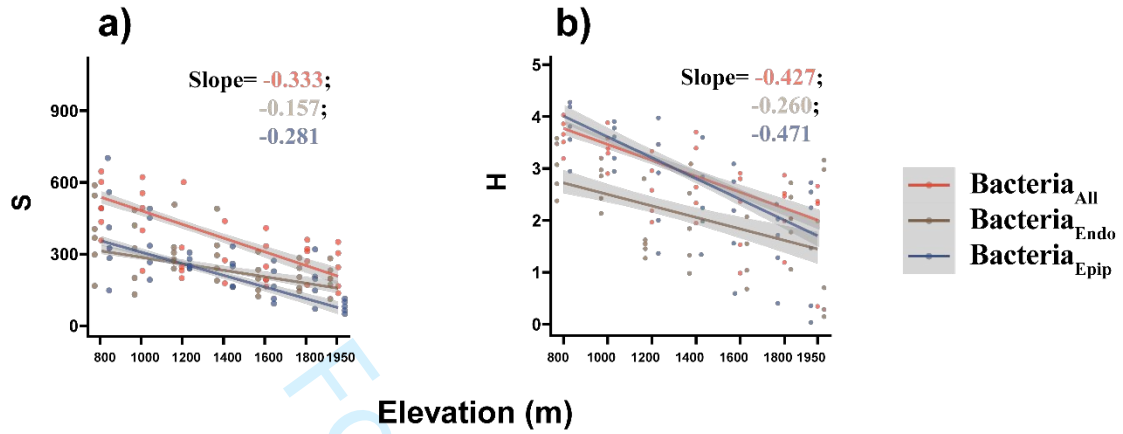
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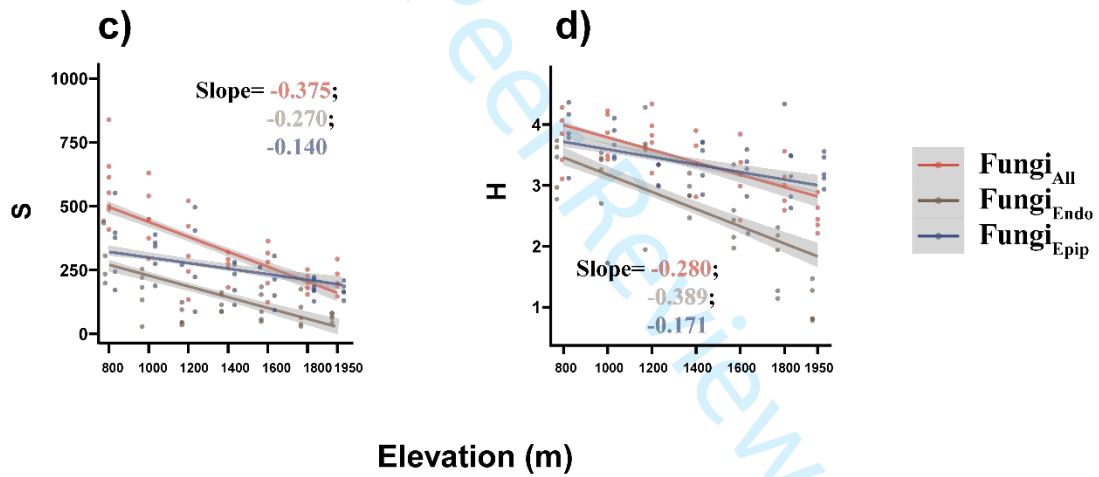
571 **Fig. 1**

575 Fig. 2

Bacteria



Fungi



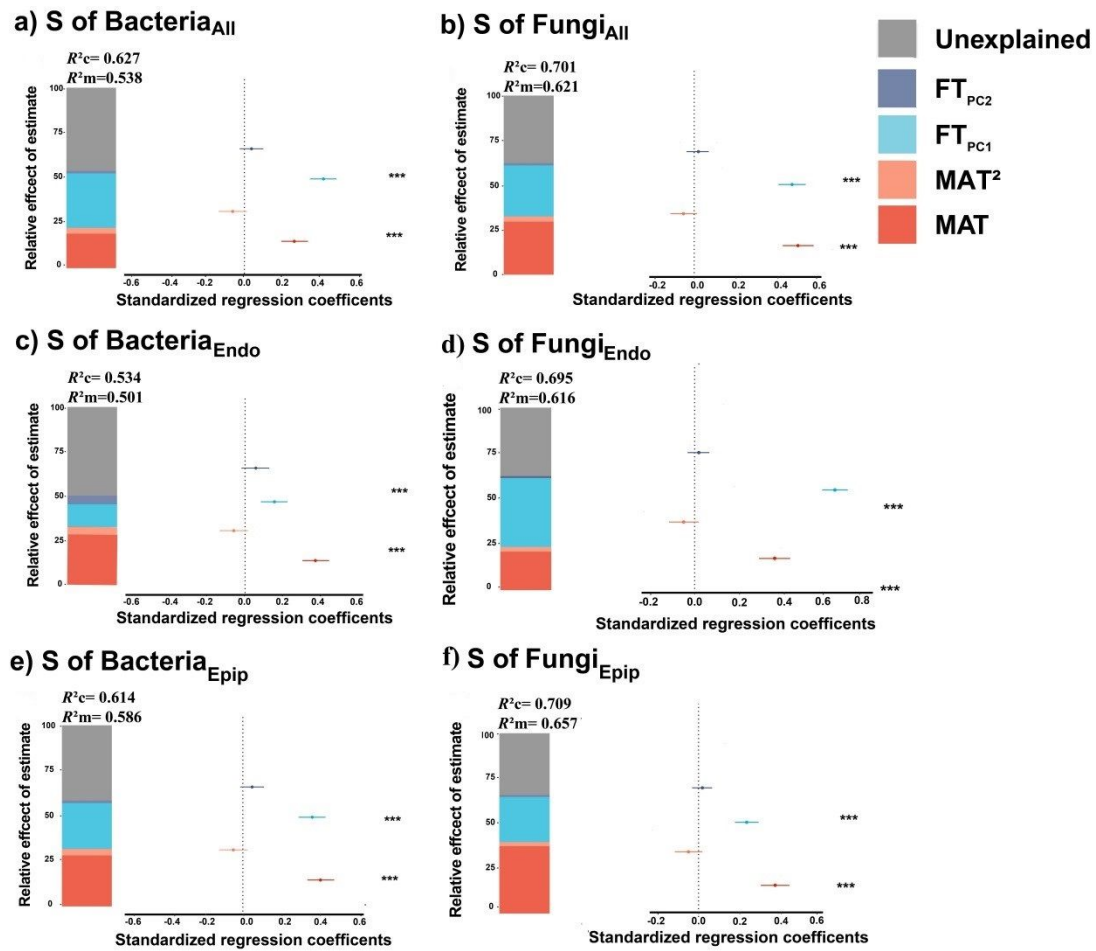
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580 Fig. 3

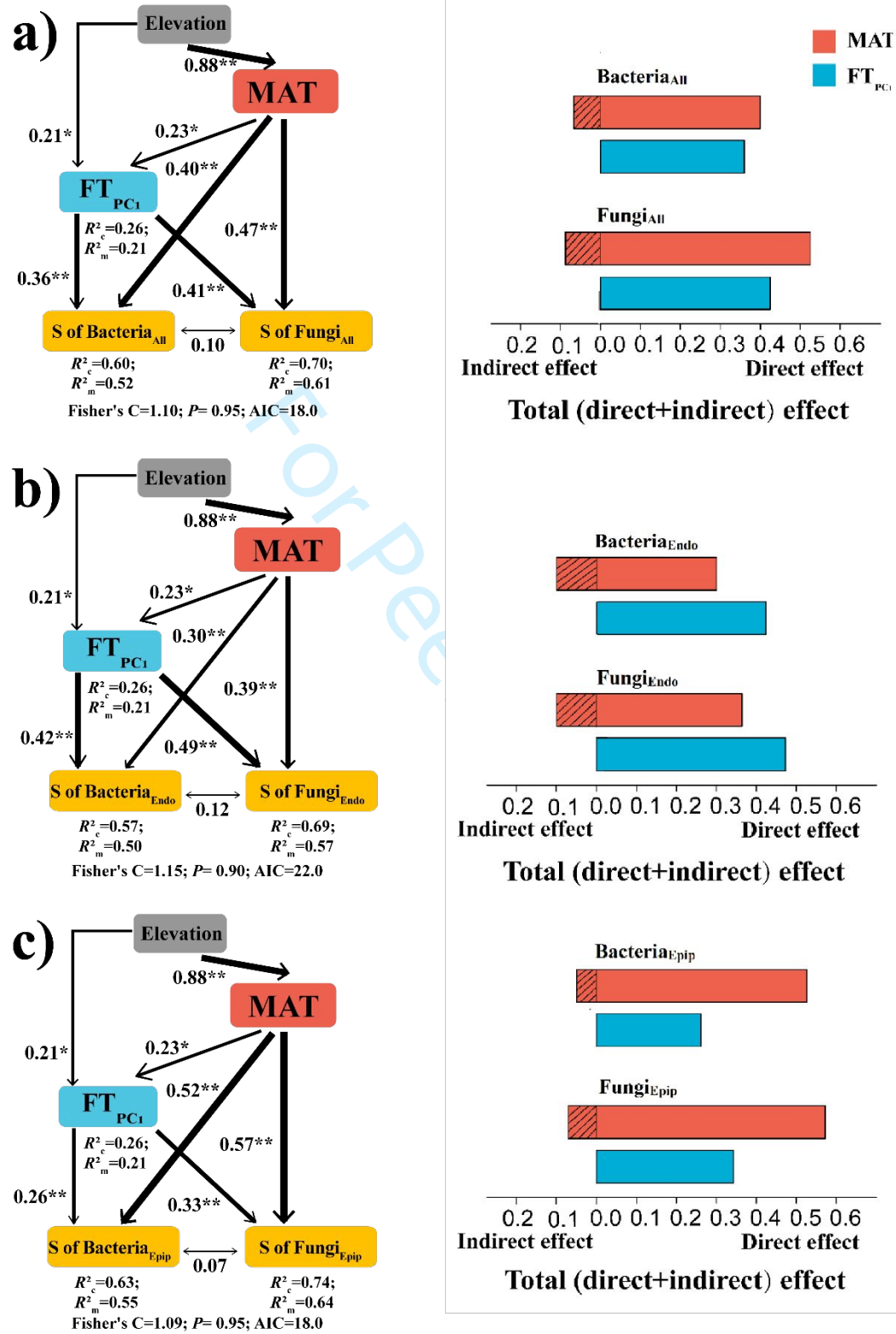


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584 Fig. 4



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Supporting information

Fig. S1 Spearman correlations among candidate predictors (the abbreviations of leaf traits were showed in Table S3)

Fig. S2 Distribution of main phyla of phyllosphere microbes (the abbreviations of tree species were shown in Table S1)

Fig. S3 Relative abundances of the dominant phylum and class for bacterial and fungal in the phyllosphere separated according to elevation categories. There were significant differences between communities at different elevations. Abbreviations: Bacteria_{All}, all bacteria; Bacteria_{Endo}, endophytic bacteria; Bacteria_{Epip}, epiphytic bacteria; Fungi_{All}, all fungi; Fungi_{Endo}, endophytic fungi; Fungi_{epip}, epiphytic fungi.

Fig. S4 A conceptual model revealing the expected links of abiotic factors (mean annual temperature) and biotic factors (leaf function traits) on phyllosphere microbial diversity.

Abbreviations: Bacteria_{All}, all bacteria; Bacteria_{Endo}, endophytic bacteria; Bacteria_{Epip}, epiphytic bacteria; Fungi_{All}, all fungi; Fungi_{Endo}, endophytic fungi; Fungi_{epip}, epiphytic fungi.

Fig. S5 Effects of mean annual temperature, plant identity(CWM_{MH}), and leaf functional traits on phyllosphere bacterial (a, c, d) and fungal (b, e, f) Shannon's diversity. We present the standardized regression coefficients of model predictors, and the associated 95% confidence intervals. We also present the relative importance of each predictor (expressed as the percentage of total variance). Significance levels are **: $P < 0.01$; ***: $P < 0.001$. Abbreviations: H, Shannon's diversity; MAT, mean annual temperature; MAT², the square of mean annual temperature; bacteria_{All}, all bacteria; Bacteria_{Endo}, endophytic bacteria; Bacteria_{Epip}, epiphytic bacteria; Fungi_{All}, all fungi; Fungi_{Endo}, endophytic fungi; Fungi_{epip}, epiphytic fungi; CWM_{MH}, the

community-weighted mean of maximum tree height; FT_{PC1} , the first PCA axis on the nine functional traits considered; FT_{PC2} , the second PCA axis.

Figure. S6 Piecewise structural equation models (pSEMs) exploring the direct and indirect effects of mean annual temperature and leaf functional traits on phyllosphere bacterial and fungi Shannon's diversity. Standardized regression coefficients and significance are given (* <0.05 , ** <0.01). The effect sizes of direct and indirect paths are also presented. Abbreviation: H, Shannon's diversity; $Bacteria_{All}$, all bacteria; $Bacteria_{Endo}$, endophytic bacteria; $Bacteria_{Epip}$, epiphytic bacteria; $Fungi_{All}$, all fungi; $Fungi_{Endo}$, endophytic fungi; $Fungi_{epip}$, epiphytic fungi; FT_{pc1} , the first PCA axis on the nine functional traits studied; MAT, mean annual temperature.

Figure. S7 Piecewise structural equation models (pSEMs) exploring the effects of mean annual temperature and leaf functional traits on phyllosphere bacterial and fungi richness. Standardized regression coefficients and significance are given (* <0.05 , ** <0.01). Abbreviation: S, richness; $Bacteria_{All}$, all bacteria; $Bacteria_{Endo}$, endophytic bacteria; $Bacteria_{Epip}$, epiphytic bacteria; $Fungi_{All}$, all fungi; $Fungi_{Endo}$, endophytic fungi; $Fungi_{epip}$, epiphytic fungi; FT_{pc1} , the first PCA axis on the nine functional traits studied; MAT, mean annual temperature.

Figure. S8 Piecewise structural equation models (pSEMs) exploring the effects of mean annual temperature and leaf functional traits on phyllosphere bacterial and fungi Shannon's diversity. Standardized regression coefficients and significance are given (* <0.05 , ** <0.01). Abbreviation: H, Shannon's diversity; $Bacteria_{All}$, all bacteria; $Bacteria_{Endo}$, endophytic bacteria; $Bacteria_{Epip}$, epiphytic bacteria; $Fungi_{All}$, all fungi; $Fungi_{Endo}$, endophytic fungi; $Fungi_{epip}$, epiphytic fungi; FT_{pc1} , the first PCA axis on the nine functional traits studied; MAT, mean annual temperature.

Figure. S9 Heatmap that shows relationship between plant functional traits and specific microbial

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3 taxa (in the phyla level).
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8 **Table S1** Summary of the main characteristics of sampling sites along an elevational gradient on
9 Changbai Mountain.
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11 **Table S2** Geographic information of the studied sites.
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13 **Table S3** Functional traits and their functions.
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15 **Table S4** Loadings of the Principal Component Analysis for trait values.
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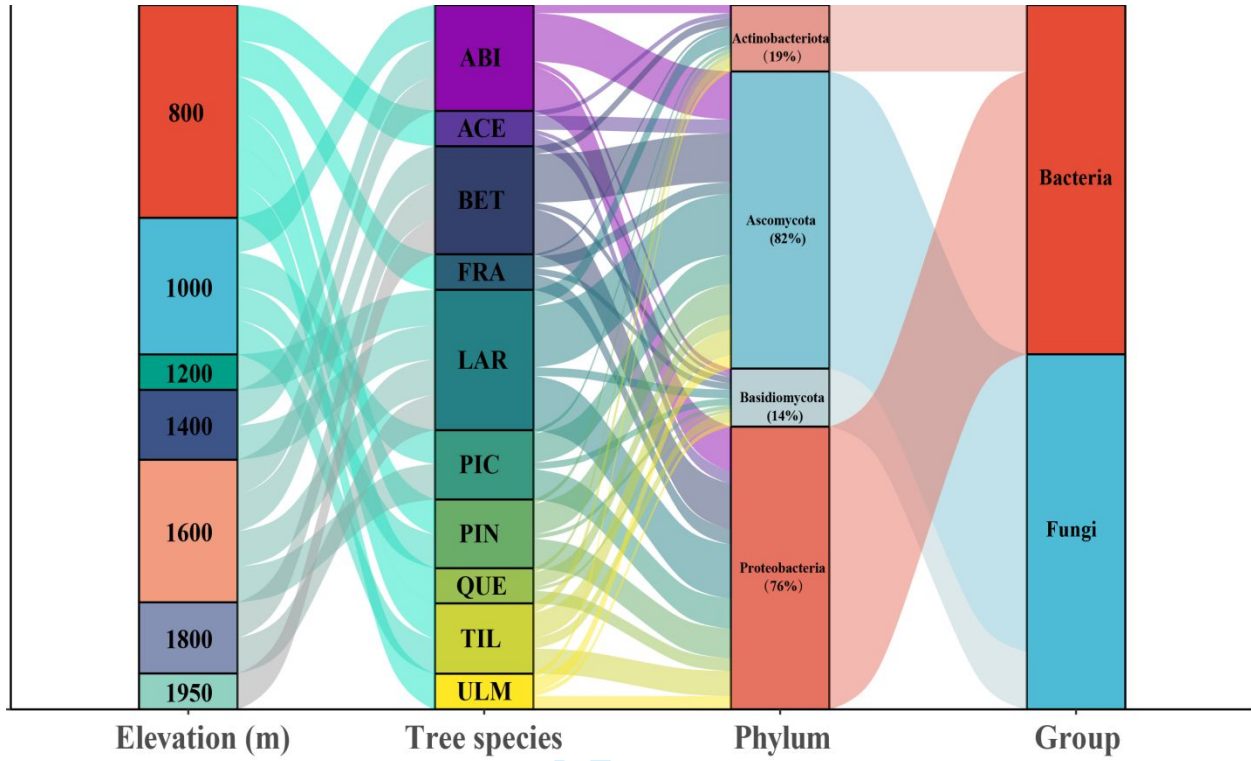
17 **Table S5** AIC value for predicting the relationship between phyllosphere microbial richness and
18 elevation(elevation²). Bold represents the optimal model based on AIC. *: $P < 0.01$.
19

20 **Table S6** AIC value for predicting the relationship between phyllosphere microbial Shannon
21 index and elevation (elevation²). Bold represents the optimal model based on AIC. *: $P < 0.01$.
22

23 **Table S7** The direct, indirect, and total standardized effects of mean annual temperature and leaf
24 functional traits on phyllosphere bacterial and fungal richness derived from the pierce structural
25 equation models. Models are presented in Fig. 4. Only significant effects are shown.
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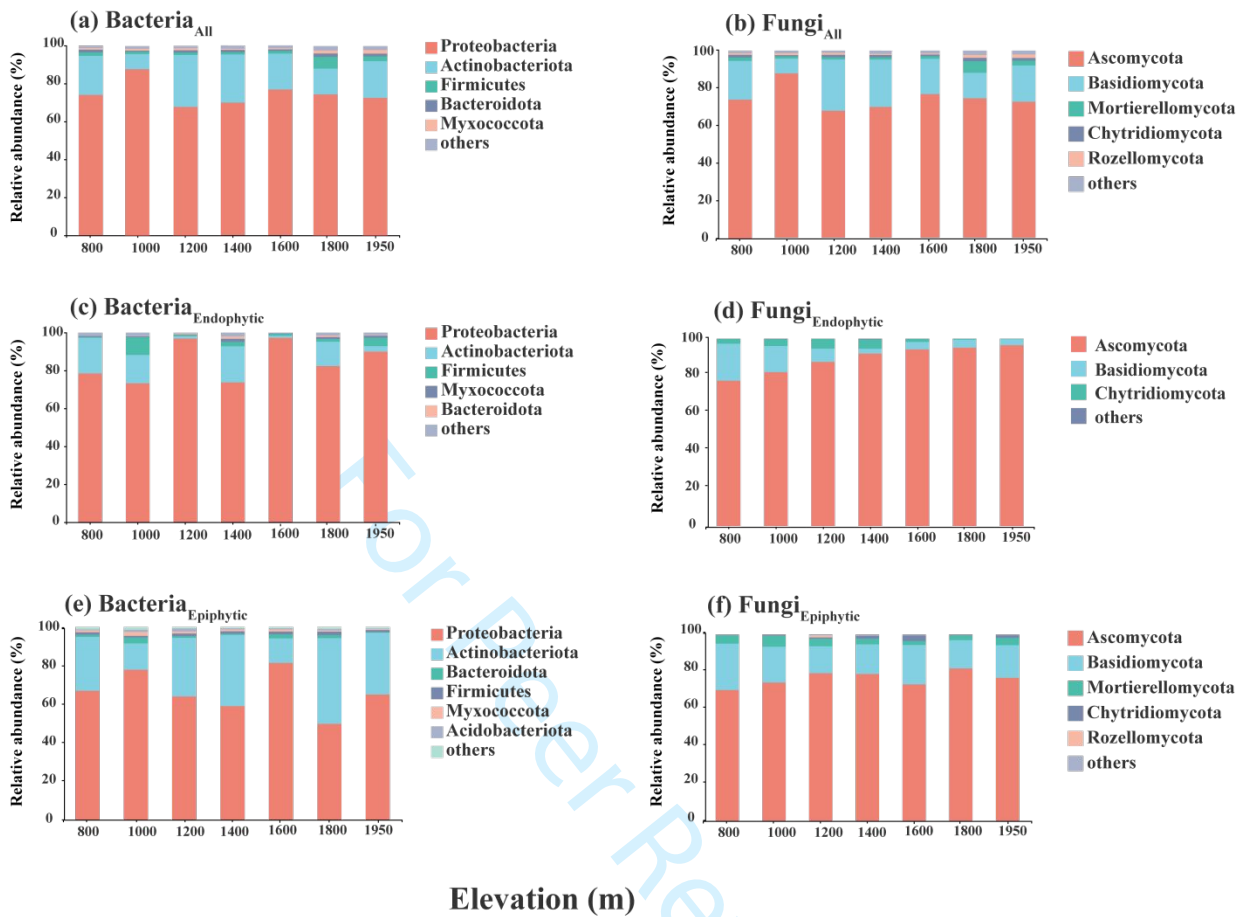
27 **Table S8** The direct, indirect, and total standardized effects of mean annual temperature and leaf
28 functional traits on phyllosphere bacterial and fungal Shannon index derived from the pierce
29 structural equation models. Models are presented in Fig. S5. Only significant effects are shown.
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Fig. S2



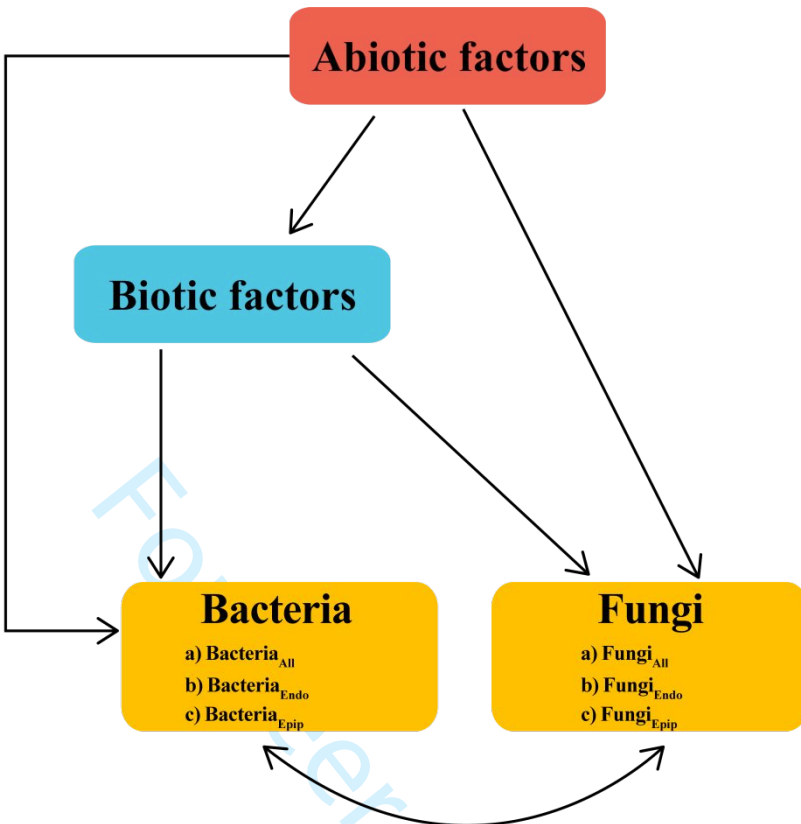
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Fig. S3



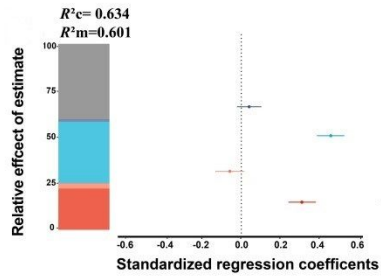
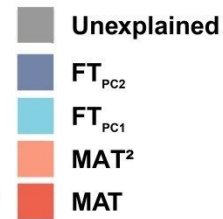
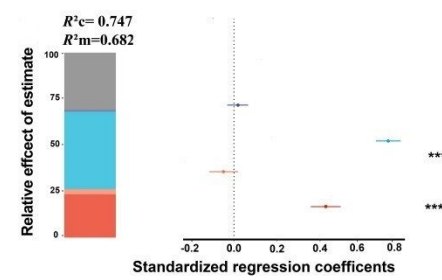
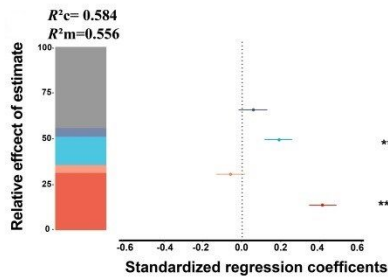
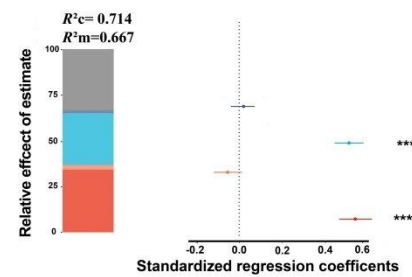
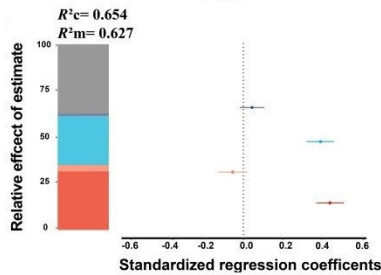
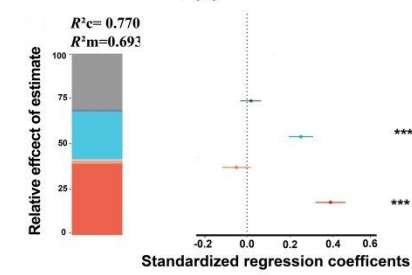
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Fig. S4



Pre-Review

Fig. S5

a) H of Bacteria_{All}b) H of Fungi_{All}c) H of Bacteria_{Endo}d) H of Fungi_{Endo}e) H of Bacteria_{Epip}f) H of Fungi_{Epip}

view

Fig. S6

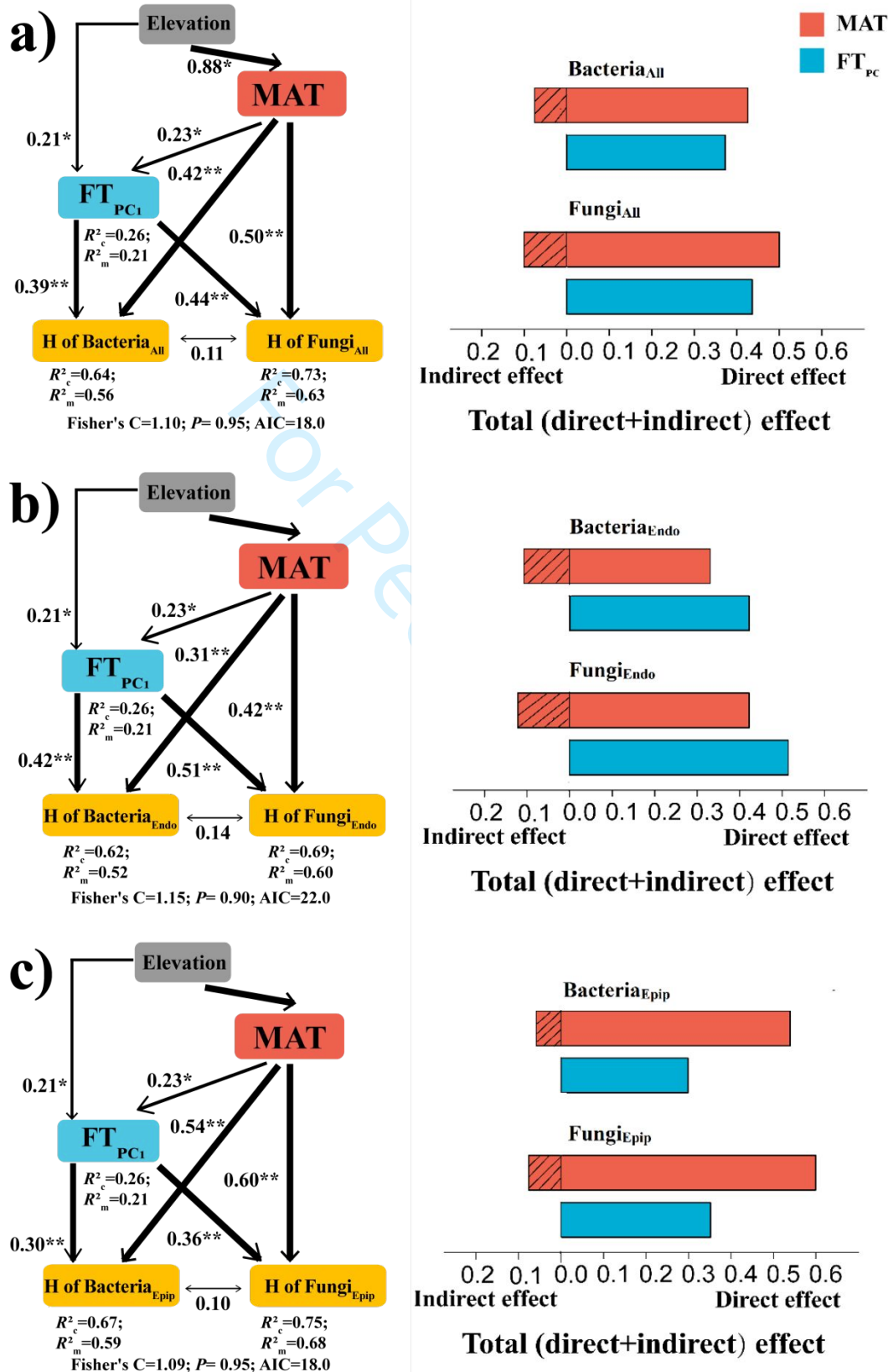


Fig. S7

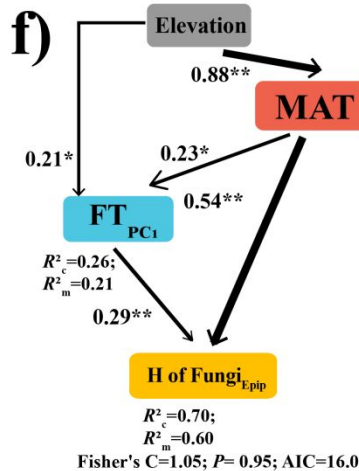
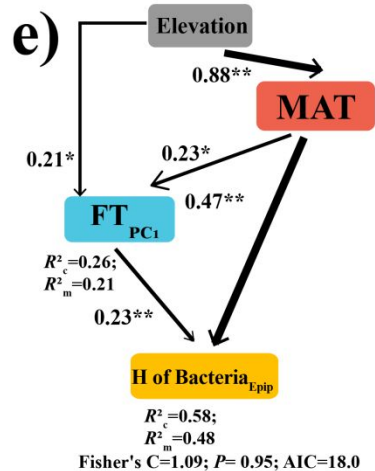
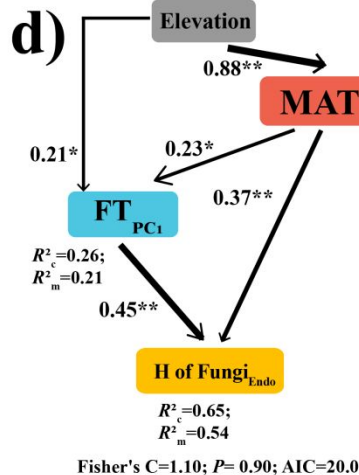
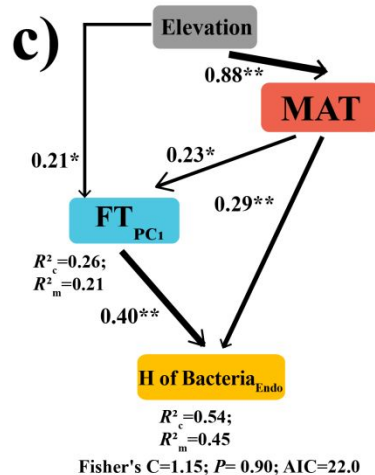
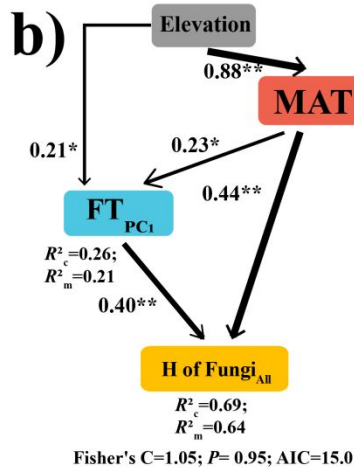
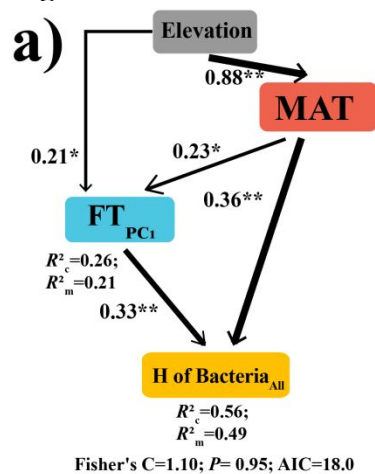


Fig. S8

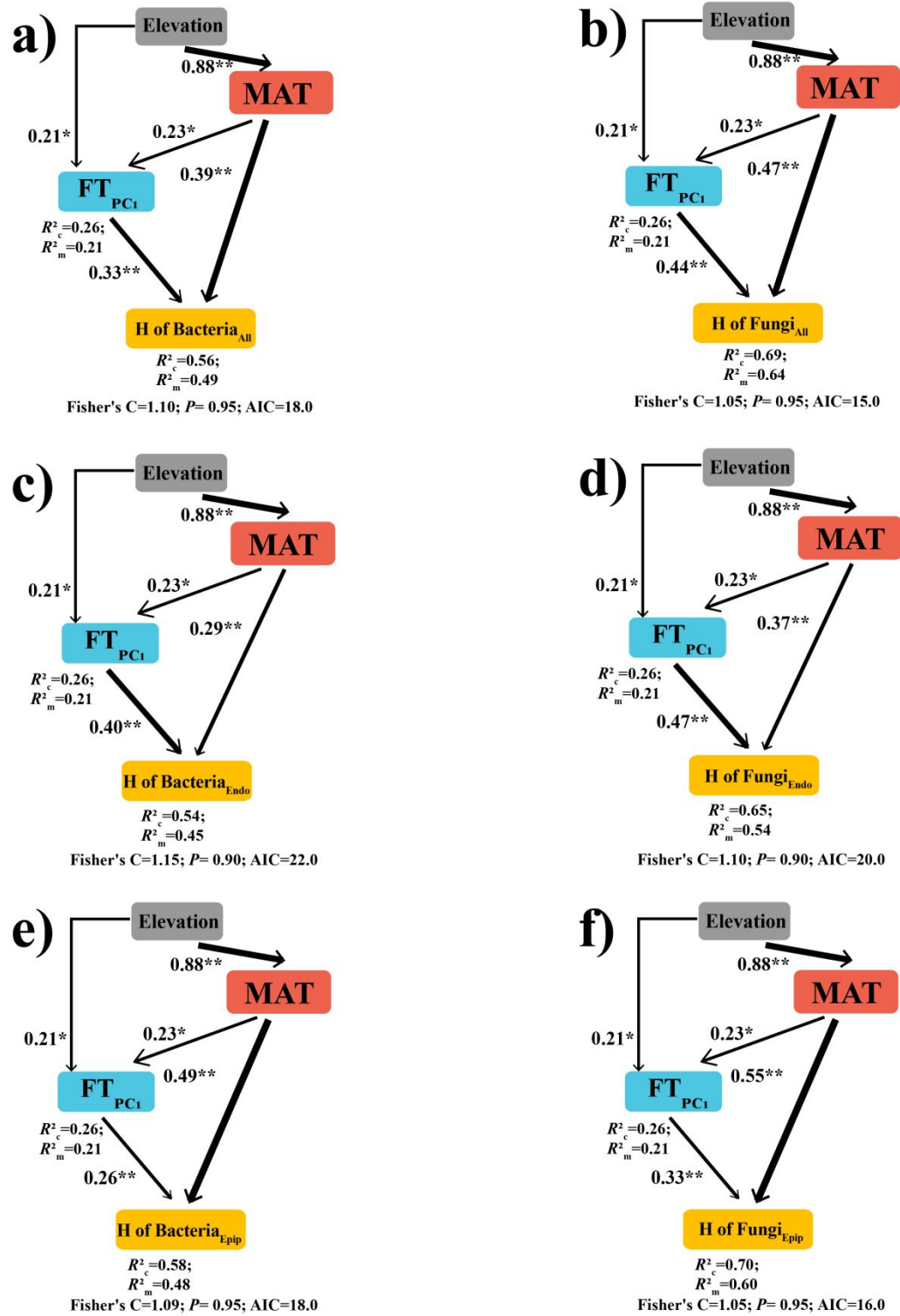
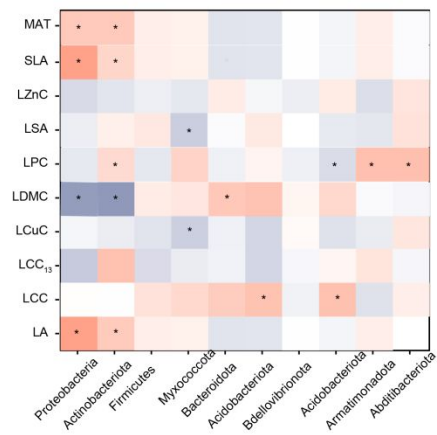
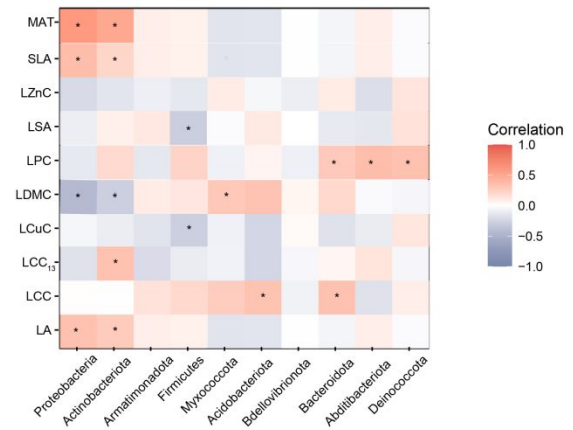


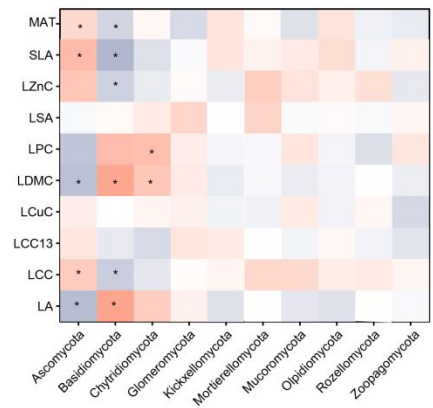
Fig. S9
a)



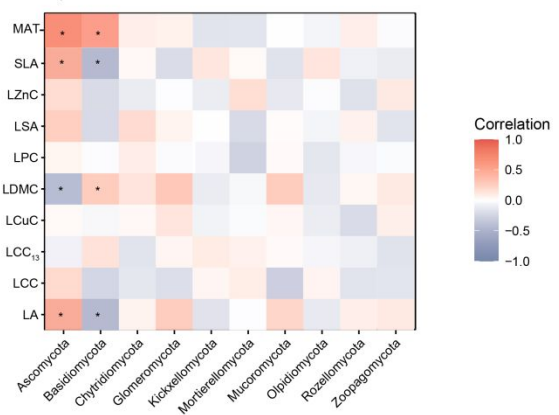
b)



c)



d)



view

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Table S1

Elevation	Vegetation type	Tree species name	Abbreviation
800m	Mixed coniferous– broadleaf forest	<i>Ulmus_japonica</i>	ULM
		<i>Quercus_mongolica</i>	QUE
		<i>Acer_mono</i>	ACE
		<i>Tilia_amurensis</i>	TIL
		<i>Pinus_koraiensis</i>	PIN
1000m	Mixed coniferous– broadleaf forest	<i>Fraxinus_mandshurica</i>	FRA
		<i>Pinus_koraiensis</i>	PIN
		<i>Tilia_amurensis</i>	TIL
		<i>Abies_nephrolepis</i>	ABI
1200m	Coniferous forest	<i>Picea_jezoensis</i>	PIC
		<i>Larix_gmelinii</i>	LAR
1400m	Coniferous forest	<i>Abies_nephrolepis</i>	ABI
		<i>Larix_gmelinii</i>	LAR
1600m	Coniferous forest	<i>Betula_ermanii</i>	BET
		<i>Abies_nephrolepis</i>	ABI
		<i>Larix_gmelinii</i>	LAR
		<i>Picea_jezoensis</i>	PIC
1800m	Birch forest	<i>Larix_gmelinii</i>	LAR
		<i>Betula_ermanii</i>	BET
1950m	Birch forest	<i>Betula_ermanii</i>	BET

Table S2

Plot	Latitude	Longitude	Elevation
1	42.2124041	128.4214579	800
2	42.2111062	128.4297998	800
3	42.35094433	127.9687601	800
4	42.3509615	127.9657993	800
5	42.3515333	127.961157	800
6	42.23425683	128.1452635	1000
7	42.23435033	128.1487417	1000
8	42.2399925	128.139942	1000
9	42.235203	128.155823	1000
10	42.23456183	128.151056	1000
11	42.16324833	128.1575617	1200
12	42.1641135	128.1527838	1200
13	42.16171583	128.1496682	1200
14	42.16287717	128.1505947	1200
15	42.16145717	128.1504003	1200
16	42.1111685	128.1021245	1400
17	42.11104967	128.1034917	1400
18	42.11009183	128.1035272	1400
19	42.11084033	128.1009102	1400
20	42.11062467	128.1025682	1400
21	42.0883485	128.0724015	1600
22	42.08745117	128.0736145	1600
23	42.08791617	128.0714043	1600
24	42.08721617	128.0710297	1600
25	42.08674417	128.0724183	1600
26	42.0671645	128.0676052	1800
27	42.06625783	128.0674132	1800
28	42.06493917	128.0665682	1800
29	42.06547567	128.0670377	1800
30	42.06414983	128.067009	1800
31	42.06086367	128.0684068	1950
32	42.06011817	128.0182908	1950
33	42.0597485	128.0676098	1950
34	42.05933067	128.0679163	1950
35	42.058869	128.0683998	1950

Table S3

Abbreviations	Functional traits	Functions
LA	Leaf Area	Resource allocation capacity
SLA	Specific Leaf Area	Resource allocation capacity
LDMC	Leaf Dry Matter Content	Resource allocation capacity
LCC ₁₃	Leaf Stable Carbon 13 Content	Water utilization efficiency
LNC ₁₅	Leaf Stable Nitrogen 15 Content	Resource utilization efficiency
LCC	Leaf Carbon Content	Plant photosynthesis
LNC	Leaf Nitrogen Content	Plant photosynthesis
LPC	Leaf Phosphorus Content	Plant photosynthesis
LKC	Leaf Potassium Content	Plant photosynthesis
LCaC	Leaf Calcium Content	Plant metabolism
LMnC	Leaf Manganese Content	Plant metabolism
LCuC	Leaf Copper Content	Plant metabolism; resistance to diseases, pests and microbes
LZnC	Leaf Zinc Content	Plant metabolism; resistance to diseases, pests and microbes
LSA	Leaf Stomatal Area	Transpiration, photosynthesis

Table S4

Traits	PC1	PC2
	(46.9%)	(28.7%)
LA	0.45	
SLA	0.51	-0.28
LDMC	-0.40	
LCC	-0.40	
LPC	-0.39	-0.33
LCuC		0.592
LZnC		0.649
LCC ₁₃		
LSA		

Table S5

	Predictors	AIC	Likelihood ratio test (Elevation vs. Elevation²)
All bacterial richness	Elevation	234.673	< 0.001
	Elevation ²	274.379	
Endophytic bacterial richness	Elevation	224.673	< 0.001
	Elevation ²	254.142	
Epiphytic bacterial richness	Elevation	227.824	< 0.001
	Elevation ²	265.015	
All fungal richness	Elevation	183.221	< 0.001
	Elevation ²	202.594	
Endophytic fungal richness	Elevation	179.002	< 0.001
	Elevation ²	194.556	
Epiphytic fungal richness	Elevation	184.017	< 0.001
	Elevation ²	199.754	

Table S6

	Predictor	AIC	Likelihood ratio test
	s		(Elevation vs. Elevation²)
All bacterial Shannon index	Elevation	227.606	< 0.001
	Elevation ²	256.386	
Endophytic bacterial Shannon index	Elevation	211.750	< 0.001
	Elevation ²	242.786	
Epiphytic bacterial Shannon index	Elevation	220.554	< 0.001
	Elevation ²	246.012	
All fungal Shannon index	Elevation	179.665	< 0.001
	Elevation ²	203.457	
Endophytic fungal Shannon index	Elevation	169.014	< 0.001
	Elevation ²	187.581	
Epiphytic fungal Shannon index	Elevation	180.171	< 0.001
	Elevation ²	188.600	

Table S7

	MAT	MAT	MAT	FT_{PC1}
	(Total)	(Direct)	(Indirect)	
All bacteria	0.476	0.395	0.081	0.358
Endophytic bacteria	0.399	0.305	0.094	0.417
Epiphytic bacteria	0.582	0.524	0.058	0.258
All fungi	0.567	0.474	0.093	0.412
Endophytic fungi	0.498	0.388	0.110	0.491
Epiphytic fungi	0.647	0.572	0.075	0.333

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Table S8 The direct, indirect, and total standardized effects of mean annual temperature and leaf functional traits on phyllosphere bacterial and fungal Shannon index derived from the piecewise structural equation models. Models are presented in Fig. 5. Only significant effects are showed.

	MAT (Total)	MAT (Direct)	MAT (Indirect)	FT_{PC1}
All bacteria	0.508	0.421	0.087	0.386
Endophytic bacteria	0.403	0.308	0.095	0.423
Epiphytic bacteria	0.603	0.535	0.068	0.304
All fungi	0.629	0.531	0.098	0.436
Endophytic fungi	0.538	0.424	0.114	0.508
Epiphytic fungi	0.684	0.603	0.081	0.359