

1 **Nutrient-rich plants emit a less intense blend of volatile**
2 **isoprenoids**

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24

25 **Abstract**

- 26 • The emission of isoprenoids (e.g. isoprene and monoterpenes) by plants
27 plays an important defensive role against biotic and abiotic stresses. Little is
28 known, however, about the functional traits linked to species-specific
29 variability in the types and rates of isoprenoids emitted and about possible
30 co-evolution of functional traits with isoprenoid emission type (isoprene
31 emitter, monoterpene emitter or both).
- 32 • We combined data for isoprene and monoterpene emission rates per dry
33 mass with key functional traits (i.e., foliar nitrogen and phosphorus
34 concentrations, leaf mass per area) and climate for 113 plant species,
35 covering the boreal, wet temperate, Mediterranean and tropical biomes.
- 36 • Foliar nitrogen was positively correlated with isoprene emission, and foliar
37 phosphorus was negatively correlated with both isoprene and monoterpene
38 emission rate. Non-emitting plants generally had the highest nutrient
39 concentrations, and those storing monoterpenes had the lowest
40 concentrations. Our phylogenetic analyses found that the type of isoprenoid
41 emission followed an adaptive, rather than a random model of evolution.
- 42 • Evolution of isoprenoids may be linked to nutrient availability and foliar
43 nitrogen and phosphorus are good predictors of the type of isoprenoid
44 emission and the rate at which monoterpenes, and to a lesser extent
45 isoprene, are emitted.

46

47 **Introduction**

48 Terrestrial ecosystems are responsible for the emission to the atmosphere of large
49 amounts of biogenic volatile organic compounds (BVOCs). BVOCs play an
50 important role in atmospheric chemistry (Carslaw *et al.*, 2009) and even climate
51 (Peñuelas & Llusia, 2003; Arneth *et al.*, 2010). Isoprenoids, including isoprene and
52 monoterpenes, are amongst the most important BVOCs emitted by plants, even
53 though not all plant species emit them (Fineschi *et al.*, 2013; Loreto & Fineschi,
54 2015). In those plants that do emit, isoprene and monoterpenes are both produced
55 during photosynthetic metabolism and can represent up to 2–5% of total
56 photosynthesis in healthy leaves, and a much higher share in stressed leaves
57 (Loreto & Schnitzler, 2010), thus potentially contributing to the carbon (C) balance
58 of ecosystems. They also have various functions in biotic and abiotic stresses such
59 as defence from herbivores or thermal and oxidative stress (Singsaas *et al.*, 1997;
60 Llusia & Penuelas, 2000; Loreto *et al.*, 2001; Peñuelas & Llusia, 2003; Penuelas &
61 Llusia, 2004; Vickers *et al.*, 2009; Niinemets, 2010).

62 Isoprenoids are important compounds for both plants and ecosystems, but why
63 emissions differ amongst species is not yet clear. Previous studies have reported a
64 trade-off between the constitutive emissions of isoprene and monoterpenes (i.e.
65 plants emitting isoprene are less likely to emit monoterpenes, or emit less)
66 (Harrison *et al.*, 2013). This trade-off should be associated with plant life histories
67 and functional traits. For example, isoprene emission is more common in woody
68 than non-woody species (Vickers *et al.*, 2009). Isoprene emission has also been
69 suggested to be more common in species from mesic than from xeric habitats, and
70 once emitted, mesic species emit at higher rates than corresponding emitting xeric
71 species. Conversely, monoterpene emission has the opposite behaviour (Loreto *et al.*
72 *et al.*, 2014a). However, a recent survey found that perennial plants of different
73 biomes share a similar fraction (around 20%) of isoprene emitters, with a
74 significantly higher emission only in deciduous plants with respect to evergreens,
75 both in temperate and tropical environments (Loreto & Fineschi, 2015).

76 Nutrient availability plays a key role in plant ecophysiology and ecosystem
77 functioning: photosynthetic rates are linked to foliar nitrogen (N) concentrations
78 (Wright *et al.*, 2004) and sometimes also to foliar phosphorus (P) (Domingues *et*
79 *al.*, 2010). Forest fruit production is linked to foliar P and zinc (Zn) concentrations
80 (Fernández-Martínez *et al.*, 2016b), whereas foliar potassium (K) is linked to
81 drought resistance (Sardans & Peñuelas, 2015). Nutrient availability is generally
82 linked to forest C sequestration (Fernández-Martínez *et al.*, 2014) and to changes
83 in the allocation of C into different plant compartments (Litton *et al.*, 2007;
84 Fernández-Martínez *et al.*, 2016a). However, it remains poorly known whether or
85 not BVOC emission types and rates in various species from different biomes
86 correlate with foliar nutrient content and the stoichiometry among key nutrients. A
87 previous study found no significant correlation between isoprenoid emissions and
88 foliar N or P concentrations, leaf mass per area (LMA), or photosynthetic capacity
89 for 70 plant species from Hawaii (Llusià *et al.*, 2010). Many studies of single
90 species, however, generally reported higher foliar N concentrations to be linked
91 with higher rates of isoprene emission (Harley *et al.*, 1994; Monson *et al.*, 1994;
92 Lerdau *et al.*, 1995; Litvak *et al.*, 1996; Staudt *et al.*, 2001; Possell *et al.*, 2004).
93 This positive relationship between isoprene emission and foliar N is consistent with
94 the observation that higher foliar N is associated to higher rates of photosynthesis
95 (Wright *et al.*, 2004), which, in turn, correlates with isoprene emission (Monson *et*
96 *al.*, 1994; Litvak *et al.*, 1996). Surprisingly, phosphorus seems to present a
97 negative relationship with isoprene emission, clearly uncoupling isoprene emission
98 from photosynthesis in *Phragmites australis* (Fares *et al.*, 2008). No direct
99 relationship was found between phosphorus and foliar volatile monoterpenes and
100 sesquiterpenes in *Pinus pinaster* (Sampedro *et al.*, 2010). The role of nutrient
101 availability in isoprenoid emission thus remains unclear.

102 Plant functional traits may account for some of the species-specific variability of
103 isoprenoid emission, but phylogenetic relationships may also play an important
104 role. Previous studies have reported a strong phylogenetic signal for isoprene but
105 not for monoterpene emissions (Llusià *et al.*, 2010; Loreto *et al.*, 2014a). This
106 strong and consistent phylogenetic signal in isoprene emission supports the

107 hypothesis that isoprene emission may have evolved in the first terrestrial plants as
108 a mechanism to cope with environmental stress and water deficit (Vickers *et al.*,
109 2009; Loreto *et al.*, 2014a). Although storage structures for terpenes are present in
110 several phylogenetically old plant groups including gymnosperms, non-storage
111 stress-dependent emissions might have evolved much more recently. Some
112 studies have analysed the emission of isoprenoids in various taxa and discussed
113 the emission type of their ancestors (Loreto *et al.*, 1998; Loreto, 2002). Recently, a
114 broad reconstruction of the ancestral emission type of a large array of
115 phylogenetically distant species has been attempted (Li *et al.*, 2017). However, the
116 mode of evolution of isoprenoids (e.g. Brownian motion [random evolution] vs.
117 adaptive mode linked to functional traits [evolution has pushed species towards
118 optimal values for adaptation] see Lapiedra *et al.*, (2013), Watson *et al.*, (2014) and
119 Sayol *et al.*, (2016) for examples), has not yet been discerned.

120 Here we study the relationships of isoprenoid emissions with plant functional traits
121 and climate, and analyse their model of evolution by performing comparative
122 phylogenetic analyses. To do so, we gathered data from published literature on
123 isoprenoid emission for 113 plant species and classified them as: i) non-emitters
124 (NE); ii) only isoprene emitters (ISP); iii) only monoterpene emitters (MTP); iv)
125 emitters of both isoprene and monoterpenes (TWO); v) MTP that also stored
126 monoterpenes (MTPs); and vi) TWO that also stored monoterpenes (TWOs).
127 Given the role that leaf functional traits have in plant ecophysiology (e.g.
128 photosynthesis, C allocation), we hypothesised that different isoprenoid emission
129 types were associated with differences in functional traits, especially nitrogen and
130 phosphorus concentrations, and were strongly evolutionarily linked.

131 **Materials and methods**

132 Data set

133 Combining our previously published data with an extensive literature search, we
134 compiled a data set (**Table S1**) of leaf isoprene and monoterpene emissions
135 containing records for plants from four biomes (boreal, wet temperate,

136 Mediterranean, and tropical). In addition to the emission rates, we have included
137 the measurement conditions for emission rate estimation (e.g. PPF
138 [photosynthetic photon flux density], temperature, plant and leaf age, canopy
139 position, growing conditions, measurement technique) and used these data to
140 convert emission rates conducted at non-standard conditions into standardised
141 values ($\mu\text{g g}^{-1} \text{h}^{-1}$) at 30 °C and $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF following the Guenther *et*
142 *al.*, (1993) equations. Emission of monoterpenes included both de-novo emissions
143 and emissions of monoterpenes from storage structures. The list of the 113
144 species included in the database and the references from where we extracted the
145 information are shown in **Table S2**. For every species for which we had values of
146 isoprene and/or monoterpene emission, we also compiled information about the
147 species, such as geographical coordinates of sampling, and species traits, such as
148 leaf habit, whether the species was woody or herbaceous, LMA, foliar N and P
149 concentrations, and monoterpene storage. When data for LMA and/or foliar N and
150 P concentrations were missing for a given species in the reviewed literature we
151 used data derived from the TRY trait database (<http://www.try-db.org>) (Kattge *et*
152 *al.*, 2011). Foliar concentrations of N and P for those species present both in the
153 database obtained from compilation of available data and in the TRY database
154 were strongly correlated (Pearson's $R = 0.93$ for N and $R = 0.91$ for P, $P < 0.001$
155 for both). Climatic data for each location (MAT and MAP) were extracted from the
156 WorldClim database (Hijmans *et al.*, 2005). That database contains long-term
157 climate averages (1950-2000), calculated on a 30 arc-second grid.

158 We used the plant phylogeny provided by Qian & Jin, (2016) for the phylogenetic
159 analyses. The names of the species in our database were matched with those in
160 the phylogenetic tree using The Plant List database in the R package *Taxonstand*
161 (Cayuela & Oksanen, 2016).

162 Data analyses

163 *Relationships between plant functional traits and climate with emission type*

164 We first categorised each species based on their emission type as: i) non-emitters
165 (NE), considered only when isoprene and monoterpene emissions equalled zero;
166 ii) only isoprene emitters (ISP); iii) only monoterpene emitters (MTP); iv) emitters of
167 both isoprene and monoterpenes (TWO); v) MTP that also stored monoterpenes
168 (MTPs); and vi) TWO that also stored monoterpenes (TWOs). MTP and TWO
169 species produce only de-novo monoterpene emissions while MTPs and TWOs
170 species produce both, de-novo emissions and emissions from monoterpene storing
171 structures. We then determined whether foliar functional traits and the climate to
172 which the plants were exposed were correlated with emission type. We performed
173 a phylogenetic principle components analysis (PCA) following Revell (2009), using
174 leaf habit (evergreens vs. deciduous, as a dummy variable), foliar concentrations of
175 N and P, foliar N:P ratio, LMA, and climate (MAT and MAP). Phylogenetic PCA
176 differs from standard PCA in that it incorporates phylogenetic information of the
177 species, and allows extracting orthogonal axes, which are free from potential
178 phylogenetic autocorrelation. We also included as a binomial trait whether or not
179 the species was woody. We then performed one-way ANOVAs to determine
180 whether the emission types affected the values of the axes extracted by the
181 phylogenetic PCA analysis. Tukey HSD tests were performed for multiple
182 comparisons.

183 Using functional traits and climate we further tried to differentiate ISP from MTP
184 species, and MTPs from MTP species. We used binomial models including
185 phylogenetic information, run via the function *phyloglm* in the R *phylolm* package
186 (Tung Ho & Ané, 2014). Response variables were coded as 0 or 1; e.g., in the
187 model for separating ISP from MTP emitters, we coded MTP emitter plants with 0
188 and ISP emitter plants with 1. In the model separating MTPs from MTP, we coded
189 with 0 plants that do not store monoterpenes and with 1 those that store them. In
190 both cases, the predictor variables were leaf habit, LMA, foliar N and P
191 concentrations, foliar N:P ratio, plant woodiness, MAT, and MAP, in addition to all
192 the numerical variables also included as ln-transformed to account for potential
193 non-linearities. The final model was obtained using stepwise backwards model
194 selection, beginning with the full model (the model containing all possible

195 predictors). Models were further fitted using a standard general linear model to
196 determine if including phylogenetic information modified our results. The results are
197 presented as partial-residuals plots from the *visreg* (Breheny & Burchett, 2015) R
198 package.

199 *Relationship between plant functional traits and climate with emission rates*

200 We explored whether foliar traits and climate could explain the amount of isoprene
201 and monoterpene emissions while also incorporating phylogenetic information in
202 the analysis. We used the *phylolm* function in the R *phylolm* package (Tung Ho &
203 Ané, 2014). We fitted the models using isoprene and monoterpenes as response
204 variables and, as predictors, LMA, leaf nutrients (foliar N and P concentrations and
205 N:P ratio), MAT, MAP, the natural-logarithmic transformations of all previous
206 covariates to account for non-linear relationships, leaf habit (evergreens vs.
207 deciduous), and whether the species was woody. Phylogenetic models were fitted
208 optimising lambda (i.e., the strength of phylogenetic signal). The final model was
209 obtained using stepwise backwards model selection, beginning with the full model.
210 Isoprene and monoterpene emissions were transformed to natural logarithms to
211 normalise the residuals.

212 *Ancestral reconstruction of emission type and their mode of evolution*

213 We used stochastic character mapping (Nielsen, 2002; Huelsenbeck *et al.*, 2003)
214 to reconstruct ancestral transitions amongst the emission types across the
215 phylogeny. This technique reconstructs the state of the ancestors of a phylogeny
216 based on its structure and the observed traits of the current species. The ancestral
217 reconstruction was achieved using the *make.simmap* function in the *phytools* R
218 package (Revell, 2012), simulating 1000 stochastic ancestral reconstructions using
219 the “mcmc” method and specifying equal rates of transition amongst the character
220 states. This analysis also allowed us to distinguish between convergent and
221 divergent evolution of type of isoprenoid emission.

222 Finally, we tested if the inferred evolutionary trajectories in foliar N and P
223 concentrations, LMA, or their adaptation to climate were associated with BVOC

224 emission type and whether an adaptive (Ornstein–Uhlenbeck: OU) or random
225 (Brownian motion—BM) model of evolution (O’Meara *et al.*, 2006; Thomas *et al.*,
226 2006; Beaulieu *et al.*, 2012) best fits the data. We fitted generalised OU-based
227 Hansen models of continuous characters (e.g. foliar N concentration) evolving
228 under discrete selective regimes (i.e. emission type) using the *OUIwie* R package
229 (Beaulieu & O’Meara, 2016). We fitted these models using 1000 randomly
230 generated ancestral reconstructions for six types of underlying evolutionary
231 processes: i) a single-state BM model (BM1), ii) a BM model with different
232 evolutionary rates for each state (emission type) on a tree (BMS), iii) an OU model
233 with a single optimal value of the continuous trait for all species (OU1), iv) an OU
234 model with different optimal values but a single alpha (the strength of the pull
235 towards the optimal values of the trait) and rate of phenotypic variation around the
236 optimal value for all emission types (OUM), v) an OU model that assumed different
237 optimal values with multiple rates of phenotypic variation per emission type
238 (OUMV), and vi) an OU model that assumed different optimal values with multiple
239 alphas (OUMA). We deleted all models containing negative eigenvalues when
240 summarising our results. For OUMA models, 99% of the stochastic character maps
241 provided models with negative eigenvalues and were therefore completely
242 excluded from our results (non-sound models). We only present the results of the
243 best types of models based on the average second-order Akaike information
244 criterion (AICc) amongst all sound models. Emission types were considered
245 significantly different when the 2.5 and 97.5% confidence intervals of two
246 categories did not overlap. All analyses used the 113 species for which we had
247 data for BVOC emissions, foliar nutrient concentrations, LMA, and climate.

248 **Results**

249 *Correlations of plant functional traits and climate with emission types and rates*

250 The first two axes extracted from the phylogenetic PCA identified significant
251 differences amongst emission types (**Figure 1**). Together they explained 49.6% of
252 the variance of the functional traits and climate. Variables most strongly aligned
253 with PC1 (**Table S3**) were LMA (positively), foliar N and foliar P (both negatively),

254 and whether a species was evergreen or deciduous (evergreens having higher
255 LMA and lower foliar nutrient concentrations). Both mean annual temperature
256 (MAT) and foliar N:P ratio were positively associated to PC1, but more weakly than
257 these other traits. PC2 was most strongly correlated with foliar P concentration
258 (positively) and N:P ratio (negatively). Additionally, PC2 was positively correlated
259 with LMA (evergreens) and negatively with MAT.

260 Both axes mainly separated the species that do (MTPs, TWOs) and do not store
261 monoterpenes (NE, MTP, ISP, TWO) (ANOVA; PC1, $P < 0.001$; PC2, $P = 0.003$).
262 The analysis also found that nutrient-rich plants belonged to the types that did not
263 emit isoprenoids or emitted only either isoprene or monoterpenes (i.e. NE, ISP,
264 and MTP). The third factor extracted did not show significant differences amongst
265 emission types.

266 More detailed analyses for discriminating the emission types using
267 phylogenetically-informed binomial regressions found that plants with high foliar P
268 concentrations and low foliar N concentrations were more likely monoterpene
269 storers (MTPs or TWOs; **Figure 2**). We also found that woody plants with higher
270 foliar N:P and P concentrations were more likely ISP than MTP (**Table 1**). Models
271 with and without phylogenetic “correction” provided the same results in both cases,
272 although those including phylogenetic information better fitted our data based on
273 $\Delta AICc$ (except for MTP emission rate, in which both models were
274 undistinguishable, see **Table 1**). This fact indicates that emission type and rates
275 present a certain degree of trait conservatism that could slightly bias model
276 estimates when considering species as independent observations.

277 Our phylogenetically-informed models for predicting isoprenoid emission indicated
278 that foliar P was negatively correlated with the rates of monoterpene and isoprene
279 emissions (**Figure 2, Table 1**). Foliar N was positively correlated with ISP emission
280 rates, and a high N:P ratio was negatively correlated with monoterpene emissions.
281 Plants with higher rates of isoprene emission were more typically woody, occurred
282 at higher MAP and had (marginally) higher LMA (**Table 1**). However, when only de-
283 novo monoterpene emission was considered (removing from the analyses species

284 belonging to MTPs and TWOs emission types) only P (negatively) and MAP
285 (positively) were marginally significantly related to monoterpene emission ($P=0.065$
286 and $P=0.058$ respectively). Again, models with and without phylogenetic correction
287 led to the same conclusions. However, including phylogenetic information
288 improved model fit of all variables except for monoterpene emission.

289 *Evolutionary reconstruction and models of emission types*

290 Evolutionary reconstructions calculated using stochastic mapping provided the
291 probability that ancestral nodes in the phylogeny represented a specific emission
292 type (**Figure 3**). Our reconstruction indicated that the oldest ancestor in our
293 phylogeny was most likely to emit both isoprene and monoterpenes and also to
294 store monoterpenes (emission type TWOs). Note, however, that our database did
295 not contain bryophytes or ferns. Most of the nodes and species throughout the
296 gymnosperm clade belonged to the TWOs emission type, despite a few transitions
297 to emit (and store) only monoterpenes (emission type MTPs). Our analysis
298 suggests that angiosperms lost their ability to store monoterpenes at some time
299 during their evolution, but a few clades later reacquired it (e.g. family *Lamiaceae*,
300 genus *Eucalyptus*). This suggests a clear case of divergent evolution (i.e.,
301 diversification of the trait through evolution) from the gymnosperms which was
302 likely associated to the evolution of different storage organs (e.g., oil glands).
303 Variability in emission type increased substantially during the diversification of
304 angiosperms, which interfered with the reconstruction of several angiosperm nodes
305 in our phylogeny. Our analysis nonetheless found that some clades had well-
306 defined ancestors (in terms of isoprenoid emission types). Species of *Salicaceae*
307 and *Fagaceae* were either in the ISP or TWO groups, and species of *Betulaceae*
308 were mainly emitters of monoterpenes only. The complete loss of the ability to emit
309 isoprene or monoterpenes was uncommon in our phylogeny, although the analysis
310 indicated some NE nodes. Having a larger number of annual plants would have
311 likely increased the number of NE nodes given that they have been suggested to
312 be non-emitters (Loreto & Fineschi, 2015).

313 An adaptive model (OU, Ornstein–Uhlenbeck model (Beaulieu *et al.*, 2012)), which
314 assumed a different optimum for each emission type and a different phenotypic
315 variability around each optimum, was the best evolutionary model explaining the
316 link between emission type and foliar concentrations of N and P, LMA, and climate
317 (MAP). For MAT, the best evolutionary model (OU) also assumed different optimal
318 MATs for each emission type, but equal phenotypic variabilities. Brownian motion
319 (BM) models always had a higher AICc (were less supported) than the OU models
320 (**Table S4**). The fact that OU models fitted data better than BM models indicates
321 that species with different emission types have most likely been pushed towards
322 optimal values (i.e., average values for a specific trait) of the variables throughout
323 evolutionary history.

324 Optimal foliar N concentrations were highest for the emission types that did not
325 store monoterpenes and were especially high for isoprene emitters only (**Figure 4,**
326 **Table 2**). In contrast, foliar P concentration was highest for non-emitters and
327 lowest for TWO and TWOs species, while ISP, MTP, and MTPs species had
328 intermediate concentrations. Differences in the optimal N:P ratio per emission type,
329 however, were not as clear; the optimal ratio was only significantly higher for TWO
330 species compared to MTPs and TWOs species, and the other types could not be
331 differentiated from any of these groups. Also, ISP species presented large
332 variability for both N:P and LMA optimum values. LMA optimal values were higher
333 for the species that stored monoterpenes (MTPs and TWOs) and lowest for MTP
334 species. Non-emitter species had the highest optimal temperatures, followed by
335 TWO, TWOs, and MTPs species. Again, ISP species presented very large
336 variability in both MAT and MAP optimal values. MTP species had the lowest
337 optimal temperature. Precipitation did not separate the different emission types as
338 much as temperature, but optimal precipitation was higher for the TWO and TWOs
339 than for the MTPs emission type, which showed the lowest average MAP optimum.
340 In summary, our results suggest two main different strategies concerning leaf
341 functional traits and isoprenoid emission type: on one side, species storing
342 monoterpenes are located towards the lower range of N and P concentrations, and
343 higher LMA. The opposite is found for non-emitters, ISP and MTP, all of which tend

344 to have higher foliar N and P and lower LMA values. Although climate, especially
345 temperature, also separated emission types, the separation was not as clear as for
346 foliar functional traits.

347 **Discussion**

348 *The role of plant functional traits and climate in determining emission type and rate*

349 Leaf functional traits (foliar N and P, N:P ratio and LMA) were the variables that
350 better explained isoprenoid emission type, and the rate at which monoterpenes,
351 and to a lesser extent, isoprene, are emitted. However, experimental studies
352 should be carried out to further confirm that the results we found in this study did
353 not appear because of the correlation between leaf functional traits and other non-
354 considered variables. Annual climate played a clearly secondary role. This is a
355 surprising result, considering the importance of certain environmental parameters
356 (namely light and temperature) in setting the emission rates of isoprene and
357 monoterpenes (Guenther *et al.*, 1993; Jardine *et al.*, 2014, 2015). Absence of
358 climate effects is instead consistent with the finding that isoprene emitters are
359 equally distributed among biomes of the world (Loreto & Fineschi, 2015). However,
360 refining the manner in which climate is considered (e.g. using data for growing
361 seasons rather than annual means) could further help in understanding the role of
362 climate as a determinant of isoprenoid type and emission. Nonetheless, some
363 studies for other foliar traits and ecosystem processes have shown that annual
364 values usually provide the same results as extreme values or averages over the
365 growing season (Niinemets, 2013, 2015; Fernández-Martínez *et al.*, 2017).
366 Different emission types had, on average, different foliar nutrient concentrations,
367 and the relationships between foliar nutrient concentrations and the emission of
368 volatile isoprenoids differed for isoprene and monoterpene emission. Based on our
369 statistical models, species with N-rich leaves were less likely to have structures for
370 storing monoterpenes but had higher rates of isoprene emission, supporting
371 previous findings linking high leaf N contents to high rates of isoprenoid emission
372 (Harley *et al.*, 1994, 1999; Monson *et al.*, 1994; Litvak *et al.*, 1996; Staudt *et al.*,
373 2001; Possell *et al.*, 2004). Plants with higher foliar N:P ratios were more likely to

374 emit isoprene but tended to emit less monoterpenes. Our results thus suggest that
375 the isoprene-monoterpene emission trade-off (Harrison *et al.*, 2013) might be
376 associated to different strategies of N use and uptake. In other words, N seems to
377 be important for emitting isoprene but not for emitting monoterpenes (Litvak *et al.*,
378 2002), albeit the relationship between isoprene emission and foliar N is not very
379 strong (**Figure 2, Table 1**). This relationship may also be due to the hydrophilous
380 nature of isoprene emitters (Loreto *et al.*, 2014a) that grow in soils in which N
381 mineralisation is not likely limited by water. These favourable environmental
382 conditions (water and nitrogen availability) enable high rates of photosynthesis
383 (Wright *et al.*, 2004) which, in turn, have been linked to high rates of isoprene
384 emission (Monson *et al.*, 1994; Litvak *et al.*, 1996). However, it is not clear whether
385 the positive correlation between foliar N and isoprene emission rate appears
386 because of a direct effect of nitrogen on isoprene emission rate or because of an
387 indirect effect through its positive effect on photosynthesis (Monson *et al.*, 1994).
388 Hence, further research on the mechanisms behind this observation is warranted.

389 Species with higher foliar P were more likely to emit isoprene and store
390 monoterpenes, but tended to emit lower rates of isoprene and monoterpenes
391 compared to those with lower foliar P concentrations (**Table 1, Figures 2 and 3**).
392 These results fully support a previous study reporting a negative correlation
393 between foliar P concentrations and isoprene emission in *Phragmites australis*,
394 suggesting that isoprene emission may not only be limited by energetic (ATP)
395 requirements (Fares *et al.*, 2008). This negative correlation between isoprene
396 emission and foliar P is puzzling because higher foliar P concentrations are also
397 related to higher photosynthetic rates (Wright *et al.*, 2004; Domingues *et al.*, 2010),
398 which are usually linked to higher isoprene emissions. One possible explanation for
399 this observation would be that high pyruvate in P-rich plants allows mitochondrial
400 respiration to more efficiently compete with the MEP pathway (Loreto *et al.*, 2007),
401 therefore inhibiting isoprene biosynthesis and emission. This is similar to what may
402 happen in plants grown under elevated CO₂ where isoprene emission is expected
403 to increase concurrently with photosynthesis, but is instead limited by competition
404 with cytosolic phosphoenolpyruvate (PEP) that is used to sustain mitochondrial

405 respiration (Rosenstiel *et al.*, 2003; Loreto *et al.*, 2007). Perhaps under high P
406 nutrition, P is mainly stored in PEP and is made unavailable for isoprene
407 biosynthesis. Alternatively, both isoprene and monoterpene emission should be
408 limited by factors other than P. Further research is clearly needed to validate these
409 hypotheses and to better determine the role of P in isoprenoid emission, given the
410 scarce literature available on this subject (Peñuelas & Staudt, 2010).

411 Plants that do not emit isoprenoids were generally within the lower range of
412 observed LMAs and the higher range of foliar N and P concentrations (and lower
413 N:P ratios), similar to what was found in previous studies on terpene content
414 (Peñuelas *et al.*, 2011). These trait types suggest that the NE plants fall towards
415 the 'fast-return' end of the leaf economic spectrum (Wright *et al.*, 2004); i.e., they
416 have potential to make faster photosynthetic returns on investments of dry mass
417 and nutrients in leaves, than species with high LMA and low N and P
418 concentrations. Conversely, MTPs and TWOs plants were within the lower range of
419 foliar nutrient concentrations and higher range of LMAs (**Table 2, Figures 1 and**
420 **4**), and so group towards the 'slow-return' end of the leaf economic spectrum. The
421 remaining groups of species (ISP, MTP and TWO) were generally found within
422 intermediate ranges of LMA and foliar nutrient concentrations. Nutrient-rich plants
423 may increase their aboveground production to the detriment of root exudates and
424 secondary metabolites (Peñuelas & Estiarte, 1998; Vicca *et al.*, 2012). As
425 secondary compounds, BVOC production may be stimulated under stress
426 conditions (e.g. stressful weather, pathogens, herbivores, or low nutrient
427 availability), i.e. when plants must invest a larger proportion of resources into
428 defence at the expense of reducing growth (Peñuelas & Estiarte, 1998; Loreto *et*
429 *al.*, 2014b). Our findings also support the hypothesis that nutrient-rich plants
430 release less carbon to the atmosphere. Therefore, our results highlight the
431 paramount role of nutrients in determining plant physiology and ecosystem
432 functioning (Elser *et al.*, 2010; Peñuelas *et al.*, 2013).

433 *Evolutionary history of isoprenoid emission type*

434 Our ancestral reconstruction suggests that the evolution of isoprenoid emission
435 type has been mainly divergent (i.e., starting from an initial trait, through evolution,
436 species develop different phenotypes amongst them) and that the primary ancestor
437 in our phylogeny was most likely to emit and store monoterpenes and emit
438 isoprene (**Figure 3**). Non emitters appear to be a minority in our database.
439 However, Loreto and Fineschi (2015) recently presented a survey of more than
440 1200 plant species showing that isoprene is emitted by around 20% of the species
441 worldwide. Thus, our database is clearly skewed toward isoprenoid-emitting plants
442 and the presence of non-emitters should be reconsidered. Emission of isoprene
443 and monoterpenes in absence of storage appears to be a second evolutionary
444 event. However, a previous study suggested that isoprene emission was
445 developed as the most primitive method to cope with heat stress, which is not a
446 problem in aquatic environments (Vickers *et al.*, 2009). Isoprene emitters are more
447 common in hygrophilous environments than in more xeric habitats, further
448 supporting this hypothesis (Loreto *et al.*, 2014a). If true, primitive terrestrial plants
449 should belong mostly to the ISP emission type, which should therefore be much
450 more common in bryophytes and pteridophytes than in vascular plants. In this
451 sense, some available reports suggest that indeed emission of isoprene is a trait
452 present in mosses (Hanson *et al.*, 1999; Lantz *et al.*, 2015) and ferns (Dani *et al.*,
453 2014). Our phylogeny, however, contained neither bryophytes nor pteridophytes,
454 and gymnosperms were the evolutionary more ancient plants in the analysis.
455 Hornworts (Anthocerotophytes) and liverworts (Marchantiophytes), however, do not
456 emit isoprene (Vickers *et al.*, 2009). Hence, future research should include these
457 older taxonomic groups, especially bryophytes, because they were the first plants
458 to colonise land, and may provide a clearer picture of the early evolutionary history
459 of isoprenoid emission type.

460 In many families of plants, species that emit isoprene can be found together with
461 non-emitters and monoterpene emitters. Previous studies suggested that a single
462 evolutionary event led to isoprene emission in early rosids, followed by multiple
463 losses (Sharkey *et al.*, 2013). For example, in the case of the oak genus, the
464 original trait (isoprene emission) may have been lost, or may have evolved into the

465 capacity to emit more complex isoprenoids (Loreto *et al.*, 1998, 2009). On the other
466 hand, several authors have embraced the hypothesis that the capacity to emit
467 isoprene was gained and lost multiple times during evolution (Loreto *et al.*, 2009;
468 Sharkey *et al.*, 2013). Li *et al.*, (2017) recently discovered that isoprene synthase
469 neo-functionalization occurs by active site mutation triggered by a single amino
470 acid mutation, supporting evolution of isoprene synthase from the large class of
471 monoterpene synthases (TPS-b). Isoprene emission may be conserved only in the
472 narrow range of environmental conditions in which it clearly benefits plant fitness
473 (Monson *et al.*, 2013), or when plant genera undergo extensive speciation, more
474 typically in perennial plants and in many trees (Dani *et al.*, 2014).

475 Our models indicated that the rate of isoprene emission had a relatively strong
476 phylogenetic signal ($\lambda = 0.51$) but the rate of monoterpene emission was poorly
477 explained by phylogeny ($\lambda = 0.14$). These results fully support previous findings for
478 phylogenetic signals in isoprene and monoterpene emission (Llusià *et al.*, 2010;
479 Loreto *et al.*, 2014a). Whether a plant belonged to the ISP or MTP emission type
480 *per se* or whether or not it stored monoterpenes, though, also had a clear
481 phylogenetic signal (**Table 1**). These results indicate that emission type (ISP or
482 MTP, monoterpene storage or not) was better preserved in the phylogeny than
483 emission rate, which is likely because specific mutations are required for isoprene
484 and monoterpene emission (and storage) and, once a species acquires them, rates
485 of emission may vary depending on the environment. The stronger phylogenetic
486 signal for isoprene than monoterpene emission, though, seems counterintuitive
487 because of the previously reported trade-off that exist between them (Harrison *et*
488 *al.*, 2013; Li *et al.*, 2017). Some authors have argued that the difference in
489 phylogenetic signal of isoprene and monoterpene emission was due to the lack of
490 ecological pressure in isoprene emission, while monoterpene emission developed
491 as an adaptation to xeric environments (Loreto *et al.*, 2014a). Our analyses,
492 though, did not attribute higher optimal values of MAP for ISP than for MTP or
493 MTPs (**Figure 4, Table 1**). However, ISP optimal values were more variable than
494 for the rest of the emission types (specially for LMA and N:P ratio, see **Figure 4**),

495 which might indicate larger variability in ISP plant traits compared to the other
496 groups.

497 In contrast, our results indicated that isoprenoid emission type evolved together
498 with foliar nutrient concentrations, LMA, and the climate they can tolerate, following
499 an adaptive rather than a random model of evolution. This finding indicates that the
500 different strategies of emission would have been selected under different specific
501 environments together with functional traits. The inability to store monoterpenes for
502 most of the angiosperms (**Figure 3**) might be due to an earlier adaptation to more
503 fertile environments than for gymnosperms, which allowed angiosperms to better
504 compete in these suitable cases. Instead, at some point of evolution,
505 gymnosperms might have developed structures to store monoterpenes that were
506 useful to tolerate stress, typically more severe in nutrient-limited environments.
507 Angiosperms may have lost or not developed this ability until some clades began
508 their adaptation to stressful environments. These clades might have developed or
509 reacquired structures to store monoterpenes (e.g., *Eucalyptus* spp., family
510 *Lamiaceae*) to better cope with stressful conditions.

511

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527 **Author contributions**

528 M.F-M. and J.P. conceived, analysed and wrote the paper. J.L., I.F., U.N., A.A.,
529 I.J.W. and F.L. provided data and contributed substantially to the writing and
530 discussion of the paper.

531 **Competing interests**

532 The authors declare no conflict of interests.

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743 **Table S1:** Dataset analysed in this study.

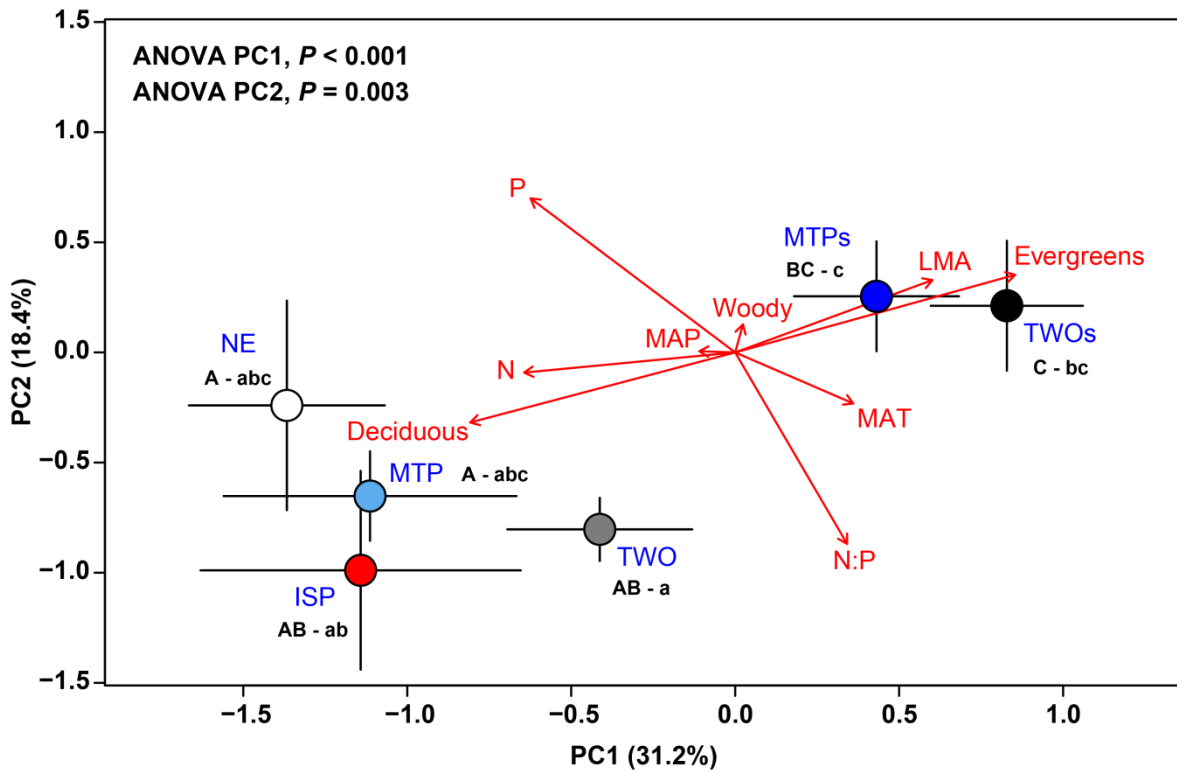
744 **Table S2:** Literature used to extract data for isoprenoid emissions.

745 **Table S3:** Loadings of the phylogenetic PCA shown in Figure 1.

746 **Table S4:** Summary statistics of the OU-based Hansen models.

747 **Figure captions**

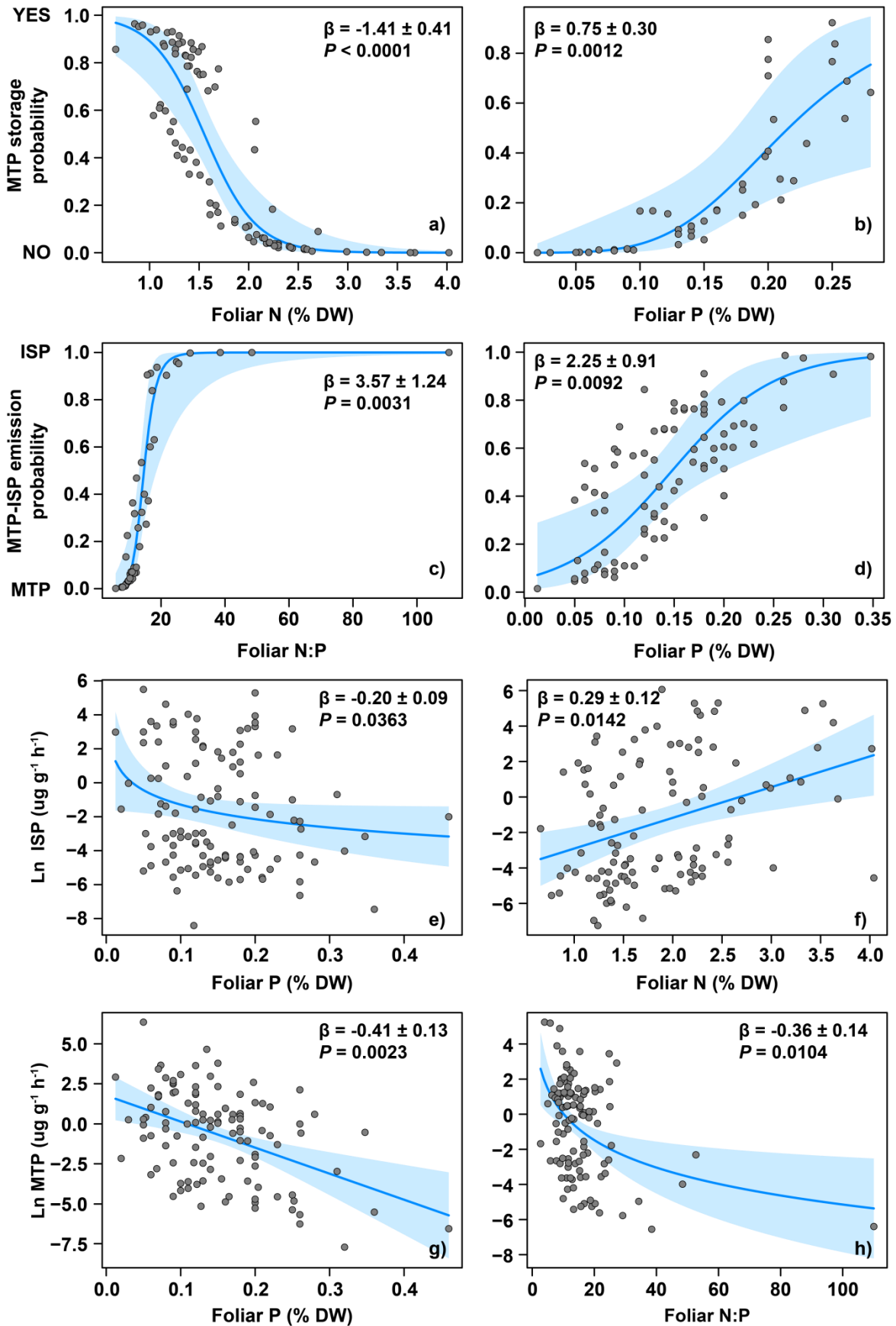
748 **Figure 1:** Average (\pm standard error) PC1 and PC2 scores per emission type: non-
749 emitters (NE), monoterpene and isoprene emitters only (MTP and ISP, respectively), and emitters of
750 monoterpenes and isoprene (TWO). MTPs and TWOs are the emission types that store monoterpenes. Different upper- and
751 lowercase letters (e.g., AB – ab) indicate statistically significant differences at the
752 0.05 level amongst emission types for the PC1 and PC2 axes, respectively, following Tukey's HSD test. Red arrows represent the loadings in the phylogenetic
753 PCA. Factor loadings can be found in **Table S3**.



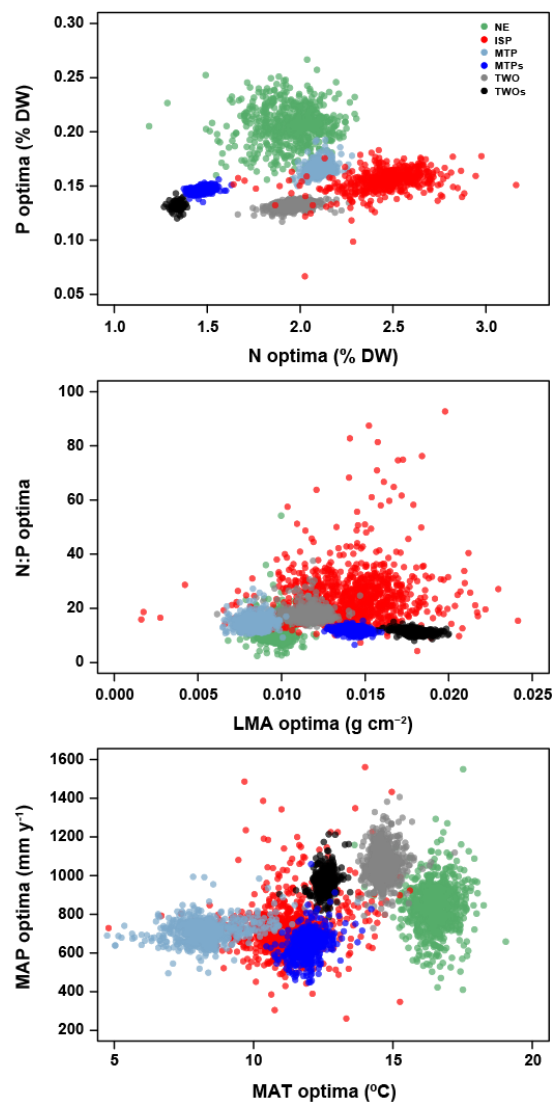
756

757

758 **Figure 2:** Partial-residual plots showing the relationships between foliar nutrient
759 concentrations of nitrogen (N) and phosphorus (P) and their stoichiometry (N:P
760 ratio) with the probability that a species stores monoterpenes (1) or not (0) **(a, b)**,
761 or that it emits either monoterpenes (0) or isoprene (1) **(c, d)**, and with emission
762 rates of isoprene **(e, f)** and monoterpenes **(g, h)**. Results of the models are
763 presented in **Table 1**. Ln indicates that the variable was log-transformed. Partial-
764 residual plots show variation in the dependent variable in relation to a given
765 predictor (the fitted line), while simultaneously controlling for all other predictors in
766 the model. The blue-shaded area indicated the 95% confidence bands of the slope
767 around the fitted line. Results of the models are presented in **Table 1**. DW, dry
768 weight.



779 **Figure 4:** Optimal values of the predictor variables for the six emission types
 780 estimated with OUMV models (Ornstein-Uhlenbeck assuming different optimal
 781 values and phenotypic variability for each emission type) for the 1000 stochastic
 782 character maps (in the graphs there is a point for every emission type and model).
 783 The results for MAT were calculated with OUM models (assuming different state
 784 means but equal multiple rates of evolution) due to lower AICc (see **Table S4**). N,
 785 foliar nitrogen concentration; P, foliar phosphorus concentration; LMA, leaf mass
 786 per area; MAT, mean annual temperature; MAP, mean annual precipitation; NE,
 787 non-emitters; ISP, isoprene emitters only; MTP, monoterpene emitters only; MTPs,
 788 monoterpene emitters only that store monoterpenes; TWO, emitters of both
 789 isoprene and monoterpenes; TWOs, emitters of both isoprene and monoterpenes
 790 that also store monoterpenes; DW, dry weight. Medians are presented in **Table 2**.



792 **Table 1:** Summary of the phylogenetically-corrected models correlating monoterpene and isoprene emissions with foliar
793 nitrogen (N) and phosphorus (P) concentrations and N:P ratio, leaf mass per area (LMA), mean annual precipitation
794 (MAP), and type of plant (woody or non-woody). See **Methods** for further information on how the models were adjusted.
795 Estimates ($\beta \pm SE$) are standardised coefficients \pm standard error. For the factor *woody*, the estimate reflects the change
796 from non-woody to woody plants. $\Delta AICc$ indicates the difference in AICc between the general linear model and the model
797 controlling for phylogeny; positive values indicate a better adjustment of the phylogenetic model. λ and α indicate the
798 phylogenetic corrections for Gaussian and binomial models, respectively. Continuous variables indicated with “Ln” were
799 log-transformed, except for models indicated with †. MTP and ISP emission rate were log-transformed to fit the models.

800

	MTP vs. ISP		MTP storage		MTP emission rate		ISP emission rate	
	Estimate	P	Estimate	P	Estimate	P	Estimate	P
Foliar N			-1.41 \pm 0.41	<0.0001			0.29 \pm 0.12	0.0142
Ln Foliar P	2.25 \pm 0.91	0.0092	0.75 \pm 0.30 [†]	0.0012	-0.41 \pm 0.13 [†]	0.0023	-0.20 \pm 0.09	0.0363
Ln Foliar N:P	3.58 \pm 1.24	0.0032			-0.36 \pm 0.14	0.0104		
LMA							0.17 \pm 0.09	0.0773
MAP							0.18 \pm 0.09	0.0345
Woody	6.75 \pm 3.36	0.0417					0.87 \pm 0.42	0.0382
$\Delta AICc$	5.13		5.13		-0.94		16.28	
λ					0.14		0.51	
α	2.85		1.22					

801

802

803 **Table 2:** Median optimal values, and their 2.5-97.5% confidence intervals, calculated with OUMV models (Ornstein-
804 Uhlenbeck, assuming evolution has pushed species towards optimal values for each emission type and to different
805 amounts of phenotypic variation around each optimum) for the six emission types. The number of sound models (i.e.,
806 without negative eigenvalues) is shown in column “N”. Different letters indicate significant differences between groups
807 based on the overlap of the confidence intervals. The results for MAT were calculated with OUM models (assuming
808 different state means but equal multiple rates of evolution) due to a lower AICc (see **Table S4**). N, foliar nitrogen
809 concentration (% dry weight); P, foliar phosphorus concentration (% dry weight); LMA, leaf mass per area (g cm⁻²); MAT,
810 mean annual temperature (°C); MAP, mean annual precipitation (mm y⁻¹); NE, non-emitters; ISP, isoprene emitters only;
811 MTP, monoterpene emitters only; MTPs, monoterpene emitters only that store monoterpenes; TWO, emitters of both
812 isoprene and monoterpenes; TWOs, emitters of both isoprene and monoterpenes that also store monoterpenes.

	NE			ISP			MTP			MTPs			TWO			TWOs		N	
	Median	CI		Median	CI		Median	CI		Median	CI		Median	CI		Median	CI		
N	1.98	2.22 1.64	c	2.48	2.76 2.06	c	2.09	2.18 2.02	c	1.49	1.54 1.41	b	1.97	2.10 1.83	c	1.35	1.37 1.32	a	782
P	0.206	0.240 0.170	c	0.156	0.172 0.132	abc	0.163	0.178 0.157	c	0.147	0.151 0.142	b	0.133	0.138 0.126	a	0.131	0.136 0.129	a	814
N:P	10.7	18.8 7.0	ab	22.8	49.2 12.9	ab	14.8	18.9 11.4	ab	11.8	13.4 10.3	a	17.5	23.0 15.1	b	11.6	12.7 9.9	a	976
LMA	0.0098	0.0107 0.0086	ab	0.0144	0.0195 0.0090	ab	0.0086	0.0094 0.0072	a	0.0144	0.0151 0.0133	c	0.0117	0.0128 0.0101	b	0.0178	0.0191 0.0170	d	979
MAT	16.5	17.6 15.4	d	11.0	12.9 9.6	ab	8.3	9.8 6.6	a	12.0	12.6 11.3	b	14.6	15.3 14.0	c	12.7	13.0 12.1	b	954
MAP	838.2	1060.7 586.3	ab	719.0	1081.1 521.4	ab	715.6	814.0 617.2	a	665.2	751.1 518.7	a	1059.2	1220.7 907.1	b	968.8	1057.5 885.6	b	875