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► To cite this version:

Philippe Janssen, Richard Chevalier, André Evette, Frédéric Archaux. The closer the better? Relative influence of forest continuity and distance to water on the taxonomic and functional structure of riparian plant communities along headwater streams. *Applied Vegetation Science*, 2021, 24 (2), pp.e12591. 10.1111/avsc.12591 . hal-03322975

HAL Id: hal-03322975

<https://hal.inrae.fr/hal-03322975v1>

Submitted on 20 Aug 2021

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1 **The closer the better? Relative influence of forest continuity and distance to water on the taxonomic and**
2 **functional structure of riparian plant communities along headwater streams**

3

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15 Running head: high value of ancient forests near stream

16

17 **Funding information**

18 Financial support was provided by the Conseil Départemental de l'Yonne (Auxerre, 89, Convention Conseil

19 Départemental de l'Yonne - Irstea n° 2016_01509 relating to the actions carried out within the framework of

20 the Protection of Sensitive Natural Spaces) and INRAE.

21 **ABSTRACT**

22 **Questions:** (1) Is forest temporal continuity an important factor in structuring the riparian plant communities
23 along small alluvial valleys? (2) To what extent can distance from the stream modulate the effect of forest
24 continuity and provide a better understanding of the taxonomic and functional structure of these
25 communities?

26 **Location:** Ouanne watershed, France.

27 **Methods:** We used a paired samples design, involving stands close and far from the stream, in ancient or
28 recent riparian forests, in which we studied the vegetation. We performed analyses of species richness and
29 cover, as well as mean values of species preferences and functional traits, and measures of species
30 composition, distinguishing between the understory and overstory.

31 **Results:** Strong differences in richness and mean values, as well as in composition, were found between
32 ancient and recent forests. Ancient forests were characterized by species with a dominant stress-tolerant
33 strategy, whereas recent forests were dominated by hygrophilous and nitrophilous species with a dominant
34 competitive strategy. Furthermore, we found that ancient and recent stands close to the stream encompassed
35 a larger richness of species, than ancient and recent riparian stands far from the stream, a pattern mostly
36 driven by the greater co-occurrence of small ruderal species with rapid leaves turnover but also of ancient
37 forest species.

38 **Conclusions:** In small riparian forest stands along headwater streams, we have shown that past human
39 activities leave still detectable traces in contemporary plant communities. In addition, we pointed out that
40 colonization by ancient indicator species was faster in recent forests closer to the stream. This underscores the
41 need to protect remaining ancient riparian forests that are well connected to the stream, even if they are small,
42 and suggests that bank reforestation as well as restoration of hydrological connectivity on rivers fragmented by
43 artificial barriers may be an effective way to increase forest biodiversity in riparian zones.

44

45 **Keywords:** ancient forests, connectivity, conservation, creek, distance to stream, forest continuity, functional
46 traits, legacy effect, riparian vegetation.

47 INTRODUCTION

48 Historical ecology has demonstrated the legacy effects that past land uses can have on the properties of
49 contemporary forest ecosystems and associated biodiversity (Peterken & Game 1984; Hermy et al. 1999; De
50 Frenne et al. 2011). Changes in land cover over time can often be deduced from historical land-use maps, such
51 as the Ordnance Survey Map in Great Britain, the Val der Maelen Map in Belgium or the État-Major Map in
52 France. With these maps, it is possible to determine precisely whether a site has been occupied by forest at
53 different times and thus to characterize the so-called ‘forest continuity’, also referred as ‘forest ancientness’ or
54 ‘ancient woodland’ (Flinn & Vellend 2005; Goldberg et al. 2007; Hermy & Verheyen 2007). More precisely, this
55 characterization makes it possible to distinguish between ancient forests, which have been forested
56 continuously for centuries, and recent forests, i.e. newly established forests resulting from spontaneous
57 afforestation on former agricultural land or artificial plantations (also known as ‘second growth’ or ‘post-
58 agricultural forests’). Forest continuity thus refers to the maintenance of forest cover over time, irrespective of
59 stand maturity and management type. It influences forest biodiversity through two mechanisms, a dispersal
60 limitation, related to increased exposure time to colonization, and a recruitment limitation, related to changes
61 in soil properties and competitive interactions (Flinn & Vellend 2005; Hermy & Verheyen 2007).

62 Research that has linked biodiversity to the temporal continuity of forest cover has mainly studied the
63 response of vascular herbaceous plants in lowland temperate forests, particularly in northwestern Europe (De
64 Frenne et al. 2011). Numerous studies have shown that the composition of plant species differed between
65 ancient and recent forests and several regional lists of ancient forest plant species have been developed (e.g.
66 Hermy et al. 1999; Matuszkiewicz et al. 2013; Bergès et al. 2016). The response of other taxa - insects (e.g.
67 Assmann 1999), lichens (e.g. Janssen et al. 2019), fungi (e.g. Spake et al. 2016) – to forest continuity has also
68 been studied, and despite variations in the magnitude and significance of the effects, have overall highlighted
69 the importance of ancient forests for conservation. However, although some studies have been carried out in
70 areas other than temperate lowland forests, as is the case in mountain and Mediterranean areas (Sciama et al.
71 2009; Janssen et al. 2018; Abadie et al. 2018), several forest types have been largely ignored in historical
72 ecology (Bergès & Dupouey 2021). This is especially the case for riparian forests, i.e., forests at the interface
73 between freshwater and terrestrial ecosystems, usually established on riverbanks.

74 Riparian forests, although representing only 1% of the European continental surface (Weissteiner et al. 2016),
75 host a unique species pool and are of critical concern for biodiversity conservation (González et al. 2017). This

76 strong ecological interest is, however, threatened by numerous anthropogenic pressures affecting riparian
77 vegetation either directly, e.g. by clearing vegetation, or indirectly, e.g. by altering the flow regime through
78 dams (González et al. 2017). Although the extent of riparian forests has increased sharply since the 1950s in
79 western Europe, due to the natural afforestation of stabilized floodplains and to large plantations of hybrid
80 poplars (Liébault & Piégay 2002; Archaux & Martin 2009), a large part of the natural riparian vegetation has
81 already been lost, degraded or fragmented. There is thus an urgent need to preserve existing intact riparian
82 vegetation and better assess the conservation value of the remaining ancient riparian forests. This is
83 particularly true in headwater streams, where the harvesting of forest stands bordering creeks may threaten
84 the quality of downstream ecosystems (Richardson & Danehy 2007; Suurkuukka et al. 2014).

85 Here, we combined a taxonomic and functional approach, to test whether forest continuity influences the
86 structure of plant communities in small remnant riparian forest stands along creeks. This double approach
87 allows both a focus on conservation issues, studying variations in species richness and identity, and a better
88 understanding of the underlying ecological processes captured by functional traits (i.e. Westoby's Leaf-Height-
89 Seed), species preferences for habitat conditions (i.e. Ellenberg's indicator values) and ecological strategies
90 deployed along disturbance and stress gradients (i.e. Grime's CSR). Based upon a dedicated sampling design in
91 which we selected ancient and recent riparian forest stands, either in close proximity to stream or far from the
92 bank, we more precisely tested whether the distance to the water changed the taxonomic and functional
93 response of plant communities to forest continuity. This has been motivated by the fact that the dispersion of
94 plant along streams is to a large part dependent on floods (Nilsson et al. 2010; Bourgeois et al. 2016), which
95 should not only favour the arrival of a higher density of propagules but also a better recruitment of species
96 (Glaeser & Wulf 2009). However, although a few studies have shown differences in the species composition of
97 ancient and recent forests in large alluvial valleys (Verheyen et al. 2003; Glaeser & Wulf 2009; Douda 2010;
98 Chevalier et al. 2014), no studies have yet focused on forest fragments bordering creeks in headwater systems
99 and none have tested the interaction between forest continuity and distance from the stream. In order to fill
100 this knowledge gap, we addressed the following two questions: (1) Is temporal continuity of forest cover an
101 important factor in the taxonomic and functional structuring of riparian plant communities in forest stands
102 along headwater streams? (2) To what extent can distance from the stream modulate the effect of forest
103 continuity and provide a better understanding of the taxonomic and functional structure of riparian plant
104 communities?

105

106 **MATERIALS & METHODS**

107 **Study area and experimental design**

108 The study was carried out along the headwater area of the Ouanne creek (size of study area watershed = 950
109 km², stream width = 2-6 m, mean annual discharge at Toucy gauging station = 0.976 m³/s) and its tributaries in
110 the center of France (Figure 1). These small alluvial valleys are under the influence of punctual and irregular
111 floods that cause disturbances of small spatial extent, i.e. often limited to riparian stands directly in contact
112 with the stream. They are characterized by a temperate climate with oceanic influences (mean annual
113 temperatures of 11.6°C and annual precipitations ranging between 700 and 800 mm) and a geological context
114 dominated by acidic silts and flint clays. To limit variation in environmental conditions, the study area was
115 restricted to the alluvial part of streams, made up of modern alluvial deposits and colonized by forests
116 dominated by European alder (*Alnus glutinosa*), field maple (*Acer campestre*), European hornbeam (*Carpinus*
117 *betulus*) and European ash (*Fraxinus excelsior*). The surrounding landscape is mainly composed of agricultural
118 areas, consisting of vast fields of crops and pastures, and wooded areas more or less connected to each other.
119 During the summers of 2018 and 2019, we sampled 24 sites in ancient (n = 12) or recent (n = 12) riparian
120 forests. For each site, we studied the vegetation in a pair of stands (n stands = 48), one directly in contact with
121 the stream, referred to as “close to stream” (ancient = 12, recent = 12), the other remote from the stream,
122 referred to as “far from stream” (ancient = 12, recent = 12) (Figure 1). Each pair was established on the same
123 stream segment and positioned in small adjacent riparian forest fragments, averaging 1.46 ha in size. The
124 distinction between stand close and far from the stream was made by establishing a buffer zone of 5 to 10 m
125 between the boundaries of the two stands where vegetation was surveyed (Figure 1). Forest continuity was
126 characterized by crossing digitized and geo-referenced 1:40,000 État-Major maps of France, charted in 1837 for
127 our study area, with 1:10,000 up-to-date forest maps (BD Forêt® V2) in a Geographic Information System (QGIS
128 Development Team 2015). Forest cover overlapping in both maps was considered to indicate ancient forests
129 (i.e. with a continuity of forest cover well above 180 years), while current forest cover overlapping with crops
130 or meadows in the État-Major maps was considered to indicate recent forests (i.e. with a continuity of forest
131 cover well below 180 years). To confirm the continuity of the forest cover since the middle of the 19th century,
132 we used a series of aerial photographs taken in 1950 at and around each site. Combined with an inspection of
133 the most recent aerial photographs available, this approach allowed us to prospect for polygons occupied by

134 forests with a natural appearance, i.e. avoiding poplar groves or harvested plots. Finally, to avoid biases due to
135 low accuracy of vegetation mapping from historical land use maps, isolated polygons < 0.25 ha and < 20 m wide
136 were eliminated.

137 **Vegetation survey**

138 At the scale of the stand (i.e. ancient-close, ancient-far, recent-close, recent-far), vegetation surveys were
139 realized following the Braun–Blanquet abundance-dominance methodology. Within a 200-m² (20 m x 10 m)
140 rectangular plot, parallel to the stream, a complete inventory was done in June/July, either in 2018 (n = 26) or
141 in 2019 (n = 22). All the vascular plants (pteridophytes and phanerogams) were recorded by considering two
142 layers, that of the overstory (i.e. tree and shrub layers ≥ 2 m height) and that of the understory (i.e. herbaceous
143 layer < 2 m height). To limit biases associated with observer effect, all surveys were performed by the same
144 experienced botanist (RC), assisted by colleagues, considering an average search effort of 30 min per plot.

145 **Stand and soil data**

146 At the scale of the site (ancient forests, n pairs = 12; recent forests, n pairs = 12), stand and soil data were
147 measured only in the stand far from the stream. This was motivated by the fact that the overstory composition
148 and structure of these riparian forests were homogeneous between stands close and far from the stream.
149 Stand attributes were characterized using a series of nested circular subplots: a 4-m-radius subplot to measure
150 trees with a diameter at breast height (DBH, at 1.3 m standard height) ≥ 5 cm, a 6-m-radius subplot for DBH ≥
151 10 cm, a 9-m-radius subplot for DBH ≥ 25 cm and a 15-m-radius subplot for DBH ≥ 45 cm. For each tree,
152 species, diameter and vitality state (alive, dying, dead), were recorded. Soil properties were characterized using
153 a soil corer in the stand center to describe the soil texture classes at 20-cm depth, based on the Jamagne's
154 (1967) texture triangle, and estimated the percentage of coarse elements ($\emptyset > 2$ mm) in the first 50 cm of soil.
155 Humus forms, i.e. mull, moder, mor and their subdivisions, were described based on three sampling points
156 located 2 m from the plot center in opposite directions. Finally, the coordinates of the stand center and the
157 elevation difference with the stream water level were measured using a GPS device and an inclinometer
158 respectively.

159 From the field measurements, we calculated a set of variables to compare environmental conditions between
160 ancient and recent forests. Stand variables (basal area of living trees and of dead trees, number of 5-cm
161 diameter classes, canopy cover percentage), were standardized to a per hectare basis and used to compare
162 overstory characteristics. Soil variables (percentage of sand/silt/clay and of coarse elements) were derived

163 either from direct measurements or from the conversion of texture classes to percentage using the central
164 value of the texture triangle. Humus forms were converted to a quantitative assessment using the humus index
165 proposed by Ponge et al. (2002). These soil variables were used to highlight possible legacy effects of past
166 human activities on edaphic conditions or pre-existing differences in environmental conditions. Spatial (latitude
167 and longitude) and topographic (altitude and elevation from water) variables were used to control for possible
168 biases in the distribution of sites within the study area.

169 **Plant preference and trait data**

170 To analyze how the ecological preferences and strategies of plants differed according to forest continuity and
171 distance from the stream, we collected data for each species from three sources of information. Ellenberg's
172 indicator values (Ellenberg et al. 1992) for light (L), nutrient availability (N) and soil moisture (F) were extracted
173 from the Baseflor database (Julve 1998) and used to characterize variations in species habitat preference.
174 Among the 194 species encountered, these values were unavailable for three species with a single occurrence
175 in our data set (i.e. singleton species) and for five taxa identified at the genus level. Grime's values (Grime
176 1977) for respectively Competitors, Stress-tolerators and Ruderals, i.e. ternary coordinates, were extracted
177 from the list of species available in Pierce et al. (2017) and used to characterize variations in the strategy–
178 environment relationships of riparian plants. For missing values (n = 54), data were either completed by using
179 ecological information on closely related species, i.e. mean value for the same genus (n = 43), or discarded
180 (genus level taxa, n = 4; singleton species, n = 2; others, n = 2). Westoby's values for specific leaf area (SLA; leaf
181 area per dry mass), plant height at maturity and seed mass were extracted from the LEDA database (Kleyer et
182 al. 2008) and used to characterize variations in resource acquisition, competitive and dispersal abilities. Missing
183 values were restricted to ten taxa (genus level taxa, n = 4; singleton species, n = 4; others, n = 2).
184 At the scale of each forest stand (n = 48), we then computed the mean values weighted by the relative cover of
185 each species bearing each value, i.e. community-weighted means (CWMs) (FD package, Laliberté et al. 2014).
186 Since herbaceous species are the most sensitive to forest continuity (Gilliam 2007) and tree species can be
187 shaped by forestry practices, CWMs were calculated only for herbaceous understory species.
188 Finally, to analyze how ancient forest species differed according to forest continuity and distance from the
189 stream, we used three local species lists of plants (Hermy et al. 1999; Dupouey et al. 2002; Bergès et al. 2016)
190 and considered as ancient forest indicator a species that appears at least twice in these lists.

191 **Statistical analysis**

192 To determine whether environmental parameters varied between ancient and recent forests (n = 24), we used
193 the Mann-Whitney U-test. For proportional data (i.e. sand, silt, clay, soil coarse elements and canopy cover),
194 logit transformation was applied prior to the calculation.

195 To determine whether species richness and cover, as well as the mean values of species' preferences and traits,
196 were influenced by forest continuity, distance to stream and their interaction, we used linear mixed models
197 (LMMs) in which "Sites" (n = 24) was included as a random effect (lme4 package, Bates et al. 2015). This
198 random effect is designed to control for the non-independence of observations made in paired stands close to
199 or far from the stream. For taxonomic measures, we used the total richness and cover of forest overstory and
200 understory species as well as the richness and cover of ancient forest indicator species as dependent variables
201 (Appendix S1). For mean values, we used as dependent variables CWMs related to species preferences (light,
202 nutrient availability and soil moisture), ecological strategies (competitors, stress-tolerators and ruderals) and
203 functional strategies of plants (SLA, height and seed mass) (Appendix S1).

204 To determine whether species composition was influenced by forest continuity, distance to the stream and
205 their interaction, we used multivariate generalized linear models (GLMs) (mvabund package, Wang et al. 2012).
206 We fitted the full model, testing the interaction between forest continuity and distance of the stream to each
207 species that was present in more than 5% of the stands, using presence-absence data, with a binomial
208 distribution and summed the likelihood ratio statistics across the univariate responses to estimate their
209 multivariate response. Because overstory composition can influence the recruitment of ancient-forest species
210 (Thomaes et al. 2014) and because tree composition can change within a few decades during riparian
211 succession (Schnitzler 1995; Fierke & Kauffman 2005; Janssen et al. 2020), we fitted one multivariate GLM for
212 the understory and one for the overstory. The significance of the factors 'forest continuity' and 'distance to
213 stream' in the multivariate GLM was assessed using an analysis of variance with the PIT-trap method and 999
214 bootstrap resamples (Warton et al. 2017). This method has the advantage of using a resampling scheme that
215 ensures that p-values are approximately correct when the independence assumption is not satisfied, as is the
216 case of our paired sampling design. To determine which herbaceous and tree species best contribute to the
217 overall model deviation, we extracted univariate test statistics and adjusted p-values for each species, using the
218 Holm's step-down procedure to correct for multiple testing (for details see, Wang et al. 2012). Finally, to
219 provide a graphical representation of the two main factors and their interaction, we used a canonical analysis

220 of principal coordinates (CAP, Anderson & Willis 2003) with the Jaccard distance community matrix (vegan
221 package, Oksanen et al. 2013).

222 Analyses were performed with R version 4.0.0 (R Core Team 2020).

223

224 **RESULTS**

225 **Variations in environmental conditions between ancient and recent riparian forests**

226 Mann–Whitney U-test revealed that only the humus index varied significantly with forest continuity, being
227 more acidic in ancient forests than in recent forests. All others environmental variables did not vary with forest
228 continuity (Appendix S2), indicating that ecological conditions were comparable between ancient and recent
229 forests in the studied area.

230 **Effect of forest continuity and distance to stream on species richness and cover and mean indicator and traits** 231 **values**

232 A total of 194 vascular plant species have been inventoried on the 48 forest stands, of which 34 species have
233 been identified as ancient forest indicator species. At stand scale, overstory richness ranged from 4 to 13
234 species (mean \pm SD = 8 ± 2.31), understory richness ranged from 14 to 58 species (mean \pm SD = 36 ± 11.9), while
235 ancient plant richness ranged from 3 to 18 species (mean \pm SD = 8 ± 3.77).

236 LMMs revealed no significant interaction between forest continuity and distance to stream (Table 1). The
237 richness and cover of ancient forest species and the mean Grime value for stress-tolerators were significantly
238 higher in ancient forest stands, while the richness and cover of overstory vegetation, the mean Ellenberg value
239 for soil moisture and nutrient availability, as well as the mean Grime value for competitors were significantly
240 higher in recent forest stands. Also, the richness of understory vegetation, the richness and cover of ancient
241 forest species, the mean Ellenberg value for nutrient availability, the mean Grime value for ruderals and the
242 mean value for SLA were significantly higher close to the stream, while the mean Grime value for stress-
243 tolerators and the mean value for plant height were significantly higher far to the stream.

244 **Effect of forest continuity and distance to stream on the species composition of overstory and understory**

245 The multivariate GLM for understory vegetation showed that species composition varied significantly with
246 forest continuity (Deviance = 259.8, $p = 0.001$) and distance to the stream (Deviance = 179.5, $p = 0.007$), but
247 not with their interaction term (Deviance = 75.9, $p = 0.617$). Based on the deviation explained by factors, most
248 of the variation in species composition was related to differences between ancient and recent forests, as

249 shown by the first CAP-axis (Figure 2-A). The second axis was more evidently related to the distance to the
250 stream. Univariate tests for each species showed that few of them contributed significantly to the multivariate
251 significant effect (Table 2). Among the species that captured the largest amount of the deviance explained by
252 forest continuity, *Angelica sylvestris* and *Filipendula ulmaria* were more frequent in recent forests, while
253 *Milium effusum* and *Carex sylvatica* were more frequent in ancient forests. For the distance to the stream,
254 species that captured the largest amount of the deviance, i.e. *Veronica montana*, *Stellaria holostea*, *Elymus*
255 *caninus* and *Phalaris arundinacea*, were all more frequent in stands close to the stream (Appendix S3).
256 The multivariate GLMs for overstorey vegetation showed that species composition varied significantly with
257 forest continuity (Deviance = 99.3, $p = 0.001$) but neither with distance to the stream (Deviance = 19.9, $p =$
258 0.643), nor with the interaction term (Deviance = 15.6, $p = 0.719$). The strong forest continuity effect is well
259 represented by the first CAP-axis (Figure 2-B), which shows clear difference in species composition between
260 ancient and recent forests. Univariate tests for each species showed that few of them contributed to the
261 multivariate significant effect. Among the trees/shrubs that captured the largest amount of the deviance
262 explained by forest continuity, *Alnus glutinosa*, *Sambucus nigra* and *Corylus avellana* were more frequent in
263 recent forests, while *Carpinus betulus* was more frequent in ancient forests (Appendix S4).

264

265 **DISCUSSION**

266 In accordance with the large amount of previous studies in European temperate forests (Hermy et al. 1999; De
267 Frenne et al. 2011; Matuszkiewicz et al. 2013; Bergès et al. 2016), we found that past land uses leave still
268 detectable traces in contemporary riparian plant communities. This highlights that even in narrow strips of
269 riparian forests along small streams, and in addition to the numerous environmental factors that structure
270 riparian communities, it is important to consider forest continuity to better understand biodiversity patterns
271 (Verheyen et al. 2003; Glaeser & Wulf 2009; Douda 2010). In addition, we showed that plant species richness,
272 including that of ancient forest indicator species, was higher in stands near the stream, and that this effect was
273 found both in ancient and recent forests. This suggests that the restoration of the hydrological connectivity on
274 rivers fragmented by artificial barriers (e.g. weirs and bed sills) could be an effective way to increase forest
275 biodiversity (Glaeser & Wulf 2009). Overall, by showing that ancient riparian forest stands, even if small in size,
276 supported distinct assemblages of plant species, our results highlight their strong ecological interest and the
277 need to better conserve the remaining fragments in agricultural landscapes.

278 **Forest continuity strongly influences riparian communities of headwater streams**

279 Our results showed that the richness and cover of overstory vegetation was higher in recent riparian forest
280 stands than in ancient ones. This may be related to the maintenance of shrubs in early successional stages
281 (Douda 2010), as confirmed by univariate analyses for each species (Appendix S4), when forest gradually
282 colonized former agricultural lands (Bergès & Dupouey 2021). This may also be related to a higher level of
283 available resources in recent forests (Hermy et al. 1999; Herault & Honnay 2005; Sciama et al. 2009), which
284 should favor a higher density of individuals and benefit a larger diversity of species (i.e. species-energy
285 hypothesis, Wright 1983). Indeed, Ellenberg's values support the view that nutrient availability was greater in
286 recent than in ancient forests, due to legacy effects of past agricultural practices or pre-existing differences in
287 soil properties, thus conditioning past land uses. Our results also showed that the richness and cover of ancient
288 forest species were significantly higher in ancient forests (Hermy & Verheyen 2007). This confirms the validity
289 of previously established lists (Hermy et al. 1999; Dupouey et al. 2002; Bergès et al. 2016), even in the riparian
290 context (Douda 2010; Chevalier et al. 2014), and, as ancient forest species represent a guild of specialists
291 sensitive to habitat loss and fragmentation (Bergès & Dupouey 2021), it pleads for priority conservation of
292 ancient forest stands, even of small size, along the banks of headwater streams. Specifically, our results
293 showed that the Grime's ecological strategies were different between ancient and recent forests, dominated
294 by stress-tolerators in the first case and by competitors in the second. Those results are consistent with
295 previous ones (Hermy et al. 1999; Hermy & Verheyen 2007; Douda 2010) and reflect a filtering of plants species
296 in relation to available environmental conditions, less limited by resources and leading to greater competitive
297 interactions between vigorous species in recent forests. However, due to overall more fertile habitat
298 conditions, it has been shown that the recruitment of ancient forest species was faster in recent forests in
299 alluvial valleys than in non-alluvial contexts (Dumortier et al. 2002; Verheyen et al. 2003). Our results tend to
300 confirm this, since of the 34 ancient forest species recorded in our study area, 26 were inventoried in recent
301 forests. This indicates that habitat conditions in recent riparian forests are globally favorable to the recruitment
302 of ancient forest species and suggests that restoration actions promoting the reforestation of riverbanks could
303 benefit forest biodiversity (McClain et al. 2011) as well as that of streams (Suurkuukka et al. 2014). Regarding
304 species composition, we found significant differences between ancient and recent forest stands. For the
305 understory, these results are consistent with those reported in temperate forests (De Frenne et al. 2011;
306 Matuszkiewicz et al. 2013; Bergès et al. 2016), including riparian forests (Verheyen et al. 2003; Glaeser & Wulf

2009; Douda 2010). As such, among the herbaceous species that predominantly occurred in ancient forests, numerous were already listed as ancient forest indicator species, e.g. *Milium effusum*, *Carex sylvatica* or *Vinca minor* (Hermy et al. 1999; Dupouey et al. 2002; Bergès et al. 2016). Interestingly, our results also emphasized plants indicative of recent forests, which are usually found in wetlands (Douda 2010), e.g. *Angelica sylvestris*, *Caltha palustris* or *Filipendula ulmaria*. Beyond showing that understory composition responds consistently to increasing soil moisture in recent forests, as expressed by Ellenberg values, our results highlight that recent forest species captured most of the explained deviance. This suggests recent forest species have a higher indicative power than ancient forest species and thus, that relevant lists of recent forest plants (Bergès et al. 2016) may be a complementary tool for identifying the continuity of forest cover in areas lacking historical land-use maps or where their accuracy is too coarse. Our results also highlighted differences in the composition of overstory. This can be attributed to different maturation stages between ancient forests, dominated by the post-pioneer species *Carpinus betulus*, and recent forests, dominated by the pioneer species *Alnus glutinosa*. Associated with changes in humus forms, these results reveal the difficulty of disentangling the effects of forest continuity from those of forest succession. Indeed, it has been shown that changes in canopy composition (Thomaes et al. 2014) and maturity stage (Janssen et al. 2018) of stands can induce a shift in herbaceous communities in ancient forests. Thus, although the difference in successional stage may partially explain the understory changes between ancient and recent forests, the fact that all other environmental variables did not vary with forest continuity supports the idea of a strong effect left by past human activities in the studied alluvial valleys.

Stream proximity enhances the conservation value of riparian forests

Total understory and ancient forest species richness was higher in stands located near the water. On the one hand, and linked to the predictions of the species-energy hypothesis (Wright 1983), this difference can be attributed to more fertile habitat conditions, as expressed by the increase in the mean Ellenberg value for nutrient availability near the stream. Indeed, in our predominantly agricultural study area, it is possible that nutrient leaching into streams preferentially enriches the best-connected adjacent riparian zones. On the other hand, this effect can be attributed to more frequent and intense floods in the stands closest to the stream. This assumption is supported by the fact that not only the richness of ruderals but also the mean value of specific leaf area increased in stands close to the stream, while the mean value of plant height decreased. Given that ruderal species are fast-growing with an annual life cycle (Grime 1977), that high SLA values indicate the

336 dominance of “acquisitive” species with rapid leaf turnover, and that smaller species indicate a shorter
337 potential growth duration between disturbances (Westoby 1998), our results provide strong evidence that
338 near-stream herbaceous communities are adapted to a more disturbed environment. Another important point
339 is related to the potential of colonization, which is known to increase along flooded areas due to the addition
340 of hydrochorous species to the local species pool (Jansson et al. 2005; Nilsson et al. 2010). However, contrary
341 to expectations, the mean value of seed mass did not increase with distance to the stream. Although seed
342 traits have been demonstrated to explain long-distance dispersal patterns (Nilsson et al. 2010; Bourgeois et al.
343 2016), local patterns of seed deposition may be less dependent on seed morphology, especially along creeks.
344 Indeed, in headwater streams, it has been shown that non-hydrochores can successfully disperse by water flow
345 (Honnay et al. 2001) but also that propagules can be caught and carried away by floating debris (Nilsson et al.
346 2010). We therefore infer that the positive effect of stream proximity on richness patterns was mainly due to
347 flooding which increased both disturbances and colonization opportunities in ancient and recent forest stands.
348 Specifically, the fact that almost all of the recent forests studied were a short distance downstream from
349 ancient forests may explain why they received more ancient forest species than stands far from the stream
350 (Honnay et al. 2001).

351 Regarding species composition, significant differences were found for understory vegetation but not for
352 overstory vegetation between stands close and far from the stream. These results thus confirm that riparian
353 forest stands belonged to the same successional stage in each pair of stands but also that distance to water
354 strongly structures herbaceous communities. Indeed, on riverbanks, the distance to the stream is most often
355 related to an elevation gradient and, by extension, to the flood gradient, well known for its great importance in
356 the structuring of riparian communities (Naiman & Decamps 1997; Poff et al. 1997). As such, numerous studies
357 have documented strong shift in species and traits composition from the bottom to the top of the riverbank
358 (Kyle & Leishman 2009; McCoy-Sulentic et al. 2017). Thus, although no differences in Ellenberg values for soil
359 moisture were found between stands close and far from the stream, in the riparian forests studied, we infer
360 that compositional changes were related to spatial variations in flood frequency and intensity as well as in
361 nutrient availability. In stands close to the stream, flooding, by periodically disturbing understory vegetation
362 but also by providing nutrients, may have favored the establishment of species requiring fertile environments
363 (e.g. *Alliaria petiolata*, *Elymus caninus*, *Phalaris arundinacea*) and/or ruderals (e.g. *Stellaria holostea*, *Veronica*
364 *montana*, *Viola reichenbachiana*).

365

366 **CONCLUSION**

367 Using a dedicated sampling design, we demonstrated that stand position relative to the stream and forest
368 continuity strongly shaped riparian plant communities, even for small wooded patches in headwater streams.
369 Specifically, we show that in fertile and humid riparian forests, the colonization credit usually documented in
370 non-alluvial settings appears to resolve more rapidly, at least for the recent forests closest to the stream. These
371 results complement well the few previous studies carried out in large alluvial valleys (Verheyen et al. 2003;
372 Hérault & Honnay 2005; Glaeser & Wulf 2009; Douda 2010) and give weight to the need to increase the
373 conservation of the remaining ancient riparian forests. Moreover, by showing that recent forests established
374 along streams were rapidly colonized by ancient forest species, our results suggest that riverbank reforestation
375 actions could be an effective way to increase the forest biodiversity. In Europe's predominantly agricultural
376 landscapes, this knowledge provides complementary arguments to encourage the conservation and restoration
377 of ancient forests. This is particularly the case along headwater streams, which account for up to 80% of the
378 total length of streams in watersheds around the world (Gomi et al. 2002).

379

380 **ACKNOWLEDGEMENTS**

381 We thank Nadia BARUCH and Antoine DELBERGUE of the CRPF Bourgogne-Franche-Comté, Marie GARNIER of
382 the IGN and Noémie BERTRAND, Adélie CHEVALIER, Barthélémy DESSANGES and Hilaire MARTIN of INRAE for
383 their help in the field and in data acquisition.

384

385 **AUTHOR CONTRIBUTIONS**

386 R.C. conceived the research idea and collected data; P.J. performed statistical analyses; P.J., wrote the first
387 draft of the paper; all authors discussed the results and commented on the manuscript.

388

389 **DATA ACCESSIBILITY**

390 The research data supporting this publication are provided in the online supporting information.

391

392 **REFERENCES**

- 393 Abadie, J., Avon, C., Dupouey, J.-L., Lopez, J.-M., Taton, T., & Bergès, L. 2018. Land use legacies on forest
394 understory vegetation and soils in the Mediterranean region: Should we use historical maps or in situ
395 land use remnants? *Forest Ecology and Management* 427: 17–25.
- 396 Anderson, M.J., & Willis, T.J. 2003. Canonical analysis of principal coordinates: a useful method of constrained
397 ordination for ecology. *Ecology* 84: 511–525.
- 398 Archaux, F., & Martin, H. 2009. Hybrid poplar plantations in a floodplain have balanced impacts on farmland
399 and woodland birds. *Forest Ecology and Management* 257: 1474–1479.
- 400 Assmann, T. 1999. The ground beetle fauna of ancient and recent woodlands in the lowlands of north-west
401 Germany (Coleoptera, Carabidae). *Biodiversity and Conservation* 8: 1499–1517.
- 402 Bates, D., Mächler, M., Bolker, B., & Walker, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of*
403 *Statistical Software* 67:.
- 404 Bergès, L., Avon, C., Arnaudet, L., Archaux, F., Chauchard, S., & Dupouey, J.-L. 2016. Past landscape explains
405 forest periphery-to-core gradient of understory plant communities in a reforestation context.
406 *Diversity and Distributions* 22: 3–16.
- 407 Bergès, L., & Dupouey, J. 2021. Historical ecology and ancient forests: Progress, conservation issues and
408 scientific prospects, with some examples from the French case. *Journal of Vegetation Science* 32:
409 e12846.
- 410 Bourgeois, B., González, E., Vanasse, A., Aubin, I., & Poulin, M. 2016. Spatial processes structuring riparian plant
411 communities in agroecosystems: implications for restoration. *Ecological Applications* 26: 2103–2115.
- 412 Chevalier, R., Archaux, F., Berthelot, A., Carnnot-Milard, L., Duprez, M., Gaudin, S., & Villemez, A. 2014. Le
413 concept de forêt ancienne s'applique-t-il aux peuplerais cultivées? Test de pertinence avec la flore des
414 vallées de Champagne. *Revue Forestière Française* LXV: 375–388.
- 415 De Frenne, P., Baeten, L., Graae, B.J., Brunet, J., Wulf, M., Orczewska, A., Kolb, A., Jansen, I., Jamoneau, A.,
416 Jacquemyn, H., Hermy, M., Diekmann, M., De Schrijver, A., De Sanctis, M., Decocq, G., Cousins, S.A.O.,
417 & Verheyen, K. 2011. Interregional variation in the floristic recovery of post-agricultural forests:
418 Recovery of post-agricultural forest. *Journal of Ecology* 99: 600–609.
- 419 Douda, J. 2010. The role of landscape configuration in plant composition of floodplain forests across different
420 physiographic areas. *Journal of Vegetation Science* 21: 1110–1124.
- 421 Dumortier, M., Butaye, J., Jacquemyn, H., Van Camp, N., Lust, N., & Hermy, M. 2002. Predicting vascular plant
422 species richness of fragmented forests in agricultural landscapes in central Belgium. *Forest Ecology*
423 *and Management* 158: 85–102.
- 424 Dupouey, J.-L., Sciama, D., Koerner, W., Dambrine, É., & Rameau, J.-C. 2002. La végétation des forêts anciennes.
425 *Revue Forestière Française* LIV: 521–532.
- 426 Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W., & Paulißen, D. 1992. Zeigerwerte von Pflanzen in
427 Mitteleuropa. *Scripta geobotanica* 18: 1–248.
- 428 Fierke, M.K., & Kauffman, J.B. 2005. Structural dynamics of riparian forests along a black cottonwood
429 successional gradient. *Forest Ecology and Management* 215: 149–162.
- 430 Flinn, K.M., & Vellend, M. 2005. Recovery of forest plant communities in post-agricultural landscapes. *Frontiers*
431 *in Ecology and the Environment* 3: 243–250.
- 432 Gilliam, F.S. 2007. The ecological significance of the herbaceous layer in temperate forest ecosystems.
433 *Bioscience* 57: 845–858.

- 434 Glaeser, J., & Wulf, M. 2009. Effects of water regime and habitat continuity on the plant species composition of
435 floodplain forests. *Journal of Vegetation Science* 20: 37–48.
- 436 Goldberg, E., Kirby, K., Hall, J., & Latham, J. 2007. The ancient woodland concept as a practical conservation
437 tool in Great Britain. *Journal of Nature Conservation* 15: 109–119.
- 438 Gomi, T., Sidle, R.C., & Richardson, J.S. 2002. Understanding processes and downstream linkages of headwater
439 systems. *BioScience* 52: 905.
- 440 González, E., Felipe-Lucia, M.R., Bourgeois, B., Boz, B., Nilsson, C., Palmer, G., & Sher, A.A. 2017. Integrative
441 conservation of riparian zones. *Biological Conservation* 211: 20–29.
- 442 Grime, J.P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological
443 and evolutionary theory. *The American Naturalist* 111: 1169–1194.
- 444 Hérault, B., & Honnay, O. 2005. The relative importance of local, regional and historical factors determining the
445 distribution of plants in fragmented riverine forests: an emergent group approach. *Journal of
446 Biogeography* 32: 2069–2081.
- 447 Hermy, M., Honnay, O., Firbank, L., Grashof-Bokdam, C., & Lawesson, J.E. 1999. An ecological comparison
448 between ancient and other forest plant species of Europe, and the implications for forest
449 conservation. *Biological Conservation* 91: 9–22.
- 450 Hermy, M., & Verheyen, K. 2007. Legacies of the past in the present-day forest biodiversity: a review of past
451 land-use effects on forest plant species composition and diversity. *Ecological Research* 22: 361–371.
- 452 Honnay, O., Verhaeghe, W., & Hermy, M. 2001. Plant community assembly along dendritic networks of small
453 forest streams. *Ecology* 82: 1691–1702.
- 454 Jamagne, M. 1967. Bases et techniques d'une cartographie des sols. *Annales agronomiques* 18: n° hors-série.
- 455 Janssen, P., Bec, S., Fuhr, M., Taberlet, P., Brun, J.-J., & Bouget, C. 2018. Present conditions may mediate the
456 legacy effect of past land-use changes on species richness and composition of above-and below-
457 ground assemblages. *Journal of Ecology* 106: 306–318.
- 458 Janssen, P., Fuhr, M., & Bouget, C. 2019. Beyond forest habitat qualities: Climate and tree characteristics as the
459 major drivers of epiphytic macrolichen assemblages in temperate mountains. *Journal of Vegetation
460 Science* 30: 42–54.
- 461 Janssen, P., Stella, J.C., Piégay, H., Rappelle, B., Pont, B., Fatou, J.-M., Cornelissen, J.H.C., & Evette, A. 2020.
462 Divergence of riparian forest composition and functional traits from natural succession along a
463 degraded river with multiple stressor legacies. *Science of The Total Environment* 721: 137730.
- 464 Jansson, R., Zinko, U., Merritt, D.M., & Nilsson, C. 2005. Hydrochory increases riparian plant species richness: a
465 comparison between a free-flowing and a regulated river. *Journal of Ecology* 93: 1094–1103.
- 466 Julve, P. 1998. Baseflor. Index botanique, écologique et chorologique de la flore de France. Version : 2017.
467 <http://perso.wanadoo.fr/philippe.julve/catminat.htm>.
- 468 Kleyer, M., Bekker, R.M., Knevel, I.C., Bakker, J.P., Thompson, K., Sonnenschein, M., Poschlod, P., van
469 Groenendael, J.M., Klimeš, L., Klimešová, J., Klotz, S., Rusch, G.M., Hermy, M., Adriaens, D., Boedeltje,
470 G., Bossuyt, B., Dannemann, A., Endels, P., Götzenberger, L., Hodgson, J.G., Jackel, A.-K., Kühn, I.,
471 Kunzmann, D., Ozinga, W.A., Römermann, C., Stadler, M., Schlegelmilch, J., Steendam, H.J.,
472 Tackenberg, O., Wilman, B., Cornelissen, J.H.C., Eriksson, O., Garnier, E., & Peco, B. 2008. The LEDA
473 Traitbase: a database of life-history traits of the Northwest European flora. *Journal of Ecology* 96:
474 1266–1274.

- 475 Kyle, G., & Leishman, M.R. 2009. Plant functional trait variation in relation to riparian geomorphology: The
476 importance of disturbance. *Austral Ecology* 34: 793–804.
- 477 Laliberté, E., Legendre, P., & Shipley, B. 2014. *FD: measuring functional diversity from multiple traits, and other*
478 *tools for functional ecology. R package version 1.0-12.*
- 479 Liébault, F., & Piégay, H. 2002. Causes of 20th century channel narrowing in mountain and piedmont rivers of
480 southeastern France. *Earth Surface Processes and Landforms* 27: 425–444.
- 481 Matuszkiewicz, J.M., Kowalska, A., Kozłowska, A., Roo-Zielińska, E., & Solon, J. 2013. Differences in plant-
482 species composition, richness and community structure in ancient and post-agricultural pine forests in
483 central Poland. *Forest Ecology and Management* 310: 567–576.
- 484 McClain, C.D., Holl, K.D., & Wood, D.M. 2011. Successional models as guides for restoration of riparian forest
485 understory. *Restoration Ecology* 19: 280–289.
- 486 McCoy-Sulentic, M.E., Kolb, T.E., Merritt, D.M., Palmquist, E., Ralston, B.E., Sarr, D.A., & Shafroth, P.B. 2017.
487 Changes in community-level riparian plant traits over inundation gradients, Colorado River, Grand
488 Canyon. *Wetlands* 37: 635–646.
- 489 Naiman, R.J., & Decamps, H. 1997. The ecology of interfaces: riparian zones. *Annual review of Ecology and*
490 *Systematics* 28: 621–658.
- 491 Nilsson, C., Brown, R.L., Jansson, R., & Merritt, D.M. 2010. The role of hydrochory in structuring riparian and
492 wetland vegetation. *Biological Reviews* 85: 837–858.
- 493 Oksanen, J., Blanchet, G.F., Kindt, R., Legendre, P., Minchin, P.R., O’Hara, R.B., Simpson, G.L., Solymos, P.,
494 Stevens, M.H.H., & Wagner, H. 2013. *vegan: Community Ecology Package. R package version 2.0-10.*
- 495 Peterken, G.F., & Game, M. 1984. Historical factors affecting the number and distribution of vascular plant
496 species in the woodlands of central Lincolnshire. *Journal of Ecology* 72: 155–182.
- 497 Pierce, S., Negreiros, D., Cerabolini, B.E.L., Kattge, J., Díaz, S., Kleyer, M., Shipley, B., Wright, S.J., Soudzilovskaia,
498 N.A., Onipchenko, V.G., van Bodegom, P.M., Frenette-Dussault, C., Weiher, E., Pinho, B.X., Cornelissen,
499 J.H.C., Grime, J.P., Thompson, K., Hunt, R., Wilson, P.J., Buffa, G., Nyakunga, O.C., Reich, P.B.,
500 Caccianiga, M., Mangili, F., Ceriani, R.M., Luzzaro, A., Brusa, G., Siefert, A., Barbosa, N.P.U., Chapin,
501 F.S., Cornwell, W.K., Fang, J., Fernandes, G.W., Garnier, E., Le Stradic, S., Peñuelas, J., Melo, F.P.L.,
502 Slaviero, A., Tabarelli, M., & Tampucci, D. 2017. A global method for calculating plant CSR ecological
503 strategies applied across biomes world-wide. *Functional Ecology* 31: 444–457.
- 504 Poff, N.L., Allan, J.D., Bain, M.B., Karr, J.R., Prestegard, K.L., Richter, B.D., Sparks, R.E., & Stromberg, J.C. 1997.
505 The natural flow regime. *BioScience* 47: 769–784.
- 506 Ponge, J.-F., Chevalier, R., & Lousot, P. 2002. Humus Index: an integrated tool for the assessment of forest
507 floor and topsoil properties. *Soil Science Society of America Journal* 66: 1996–2001.
- 508 QGIS Development Team. 2015. *QGIS Geographic Information System. Open Source Geospatial Foundation*
509 *Project.*
- 510 R Core Team. 2020. *R: A language and environment for statistical computing.* R Foundation for Statistical
511 Computing, Vienna, Austria.
- 512 Richardson, J.S., & Danehy, R.J. 2007. A synthesis of the ecology of headwater streams and their riparian zones
513 in temperate forests. *Forest Science* 53: 131–147.
- 514 Schnitzler, A. 1995. Successional status of trees in gallery forest along the river Rhine. *Journal of Vegetation*
515 *Science* 6: 479–486.

- 516 Sciama, D., Augusto, L., Dupouey, J.-L., Gonzalez, M., & Domínguez, C.M. 2009. Floristic and ecological
517 differences between recent and ancient forests growing on non-acidic soils. *Forest Ecology and*
518 *Management* 258: 600–608.
- 519 Spake, R., van der Linde, S., Newton, A.C., Suz, L.M., Bidartondo, M.I., & Doncaster, C.P. 2016. Similar
520 biodiversity of ectomycorrhizal fungi in set-aside plantations and ancient old-growth broadleaved
521 forests. *Biological Conservation* 194: 71–79.
- 522 Suurkuukka, H., Virtanen, R., Suorsa, V., Soininen, J., Paasivirta, L., & Muotka, T. 2014. Woodland key habitats
523 and stream biodiversity: Does small-scale terrestrial conservation enhance the protection of stream
524 biota? *Biological Conservation* 170: 10–19.
- 525 Thomaes, A., De Keersmaeker, L., Verschelde, P., Vandekerckhove, K., & Verheyen, K. 2014. Tree species
526 determine the colonisation success of forest herbs in post-agricultural forests: Results from a 9 yr
527 introduction experiment. *Biological Conservation* 169: 238–247.
- 528 Verheyen, K., Bossuyt, B., Honnay, O., & Hermy, M. 2003. Herbaceous plant community structure of ancient
529 and recent forests in two contrasting forest types. *Basic and Applied Ecology* 4: 537–546.
- 530 Wang, Y., Naumann, U., Wright, S.T., & Warton, D.I. 2012. mvabund - an R package for model-based analysis of
531 multivariate abundance data. *Methods in Ecology and Evolution* 3: 471–474.
- 532 Warton, D.I., Thibaut, L., & Wang, Y.A. 2017. The PIT-trap—A “model-free” bootstrap procedure for inference
533 about regression models with discrete, multivariate responses. *PLoS one* 12: e0181790.
- 534 Weissteiner, C., Ickerott, M., Ott, H., Probeck, M., Ramminger, G., Clerici, N., Dufourmont, H., & de Sousa, A.
535 2016. Europe’s Green Arteries—A Continental Dataset of Riparian Zones. *Remote Sensing* 8: 925.
- 536 Westoby, M. 1998. A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil* 199: 213–227.
- 537 Wright, D.H. 1983. Species-energy theory: an extension of species-area theory. *Oikos* 41: 496–506.

538

539 **SUPPORTING INFORMATION**

540 Appendix S1: Correlation matrix of dependent variables

541 Appendix S2: Variations of environmental variables in relation to forest continuity

542 Appendix S3: Univariate tests for understory species

543 Appendix S4: Univariate tests for overstory species

544 Appendix S5: Raw data: environmental variables

545 Appendix S6: Raw data: species by sites matrix

546 **Table 1.** Mean (\pm SD) of species richness and species cover, as well as of the community-weighted means of trait value, in relation to forest continuity (ancient vs recent) and
 547 distance to stream (close vs far) along the Ouanne creek and its tributaries, France (p-value based on linear mixed models).

Variable	Ancient forests		Recent forests		Forest	Distance	Interaction	
	close	far	close	far	continuity	to stream		
	Mean (\pm SD)	Mean (\pm SD)	Mean (\pm SD)	Mean (\pm SD)	p value	p value		
Richness and cover	Overstory richness	6.83 (\pm 1.70)	6.33 (\pm 1.92)	9.42 (\pm 1.31)	8.50 (\pm 2.78)	0.001	0.197	0.700
	Overstory cover	86.79 (\pm 20.59)	76.62 (\pm 19.99)	91.48 (\pm 17.07)	97.62 (\pm 20.03)	0.027	0.721	0.154
	Understory richness	39.25 (\pm 10.66)	28.42 (\pm 10.03)	43.67 (\pm 7.76)	34.92 (\pm 13.87)	0.161	<0.001	0.654
	Understory cover	118.05 (\pm 33.02)	109.66 (\pm 35.68)	141.57 (\pm 28.00)	126.32 (\pm 35.38)	0.087	0.134	0.656
	Ancient forest richness	11.25 (\pm 3.52)	8.42 (\pm 4.12)	8.58 (\pm 2.87)	5.42 (\pm 2.11)	0.026	<0.001	0.778
	Ancient forest cover	42.38 (\pm 21.53)	28.02 (\pm 15.23)	28.02 (\pm 22.21)	15.38 (\pm 11.57)	0.037	0.004	0.842
Mean value (CWM)	Soil moisture	5.35 (\pm 0.35)	5.19 (\pm 0.17)	5.74 (\pm 0.53)	5.81 (\pm 0.46)	0.002	0.557	0.157
	Light	4.61 (\pm 0.40)	4.66 (\pm 0.38)	4.84 (\pm 0.32)	4.95 (\pm 0.38)	0.066	0.242	0.662
	Nutrient availability	5.25 (\pm 0.82)	4.76 (\pm 1.23)	6.37 (\pm 0.71)	5.82 (\pm 0.99)	0.003	0.021	0.897
	Competitors	29.12 (\pm 5.89)	26.95 (\pm 5.45)	35.94 (\pm 6.42)	35.81 (\pm 5.06)	0.001	0.369	0.426
	Stress-tolerators	27.70 (\pm 8.83)	36.67 (\pm 16.17)	16.61 (\pm 5.98)	22.68 (\pm 10.86)	0.003	0.007	0.569
	Ruderals	43.18 (\pm 9.19)	36.38 (\pm 14.35)	47.45 (\pm 6.96)	41.52 (\pm 11.32)	0.246	0.004	0.829
	Specific leaf area	27.82 (\pm 5.80)	23.86 (\pm 6.13)	31.99 (\pm 4.03)	27.77 (\pm 6.32)	0.067	<0.001	0.892
	Plant height	0.70 (\pm 0.23)	0.94 (\pm 0.36)	0.54 (\pm 0.17)	0.77 (\pm 0.30)	0.094	0.001	0.953
	Seed mass	2.87 (\pm 1.25)	4.10 (\pm 2.49)	2.82 (\pm 1.47)	2.77 (\pm 0.94)	0.163	0.221	0.188

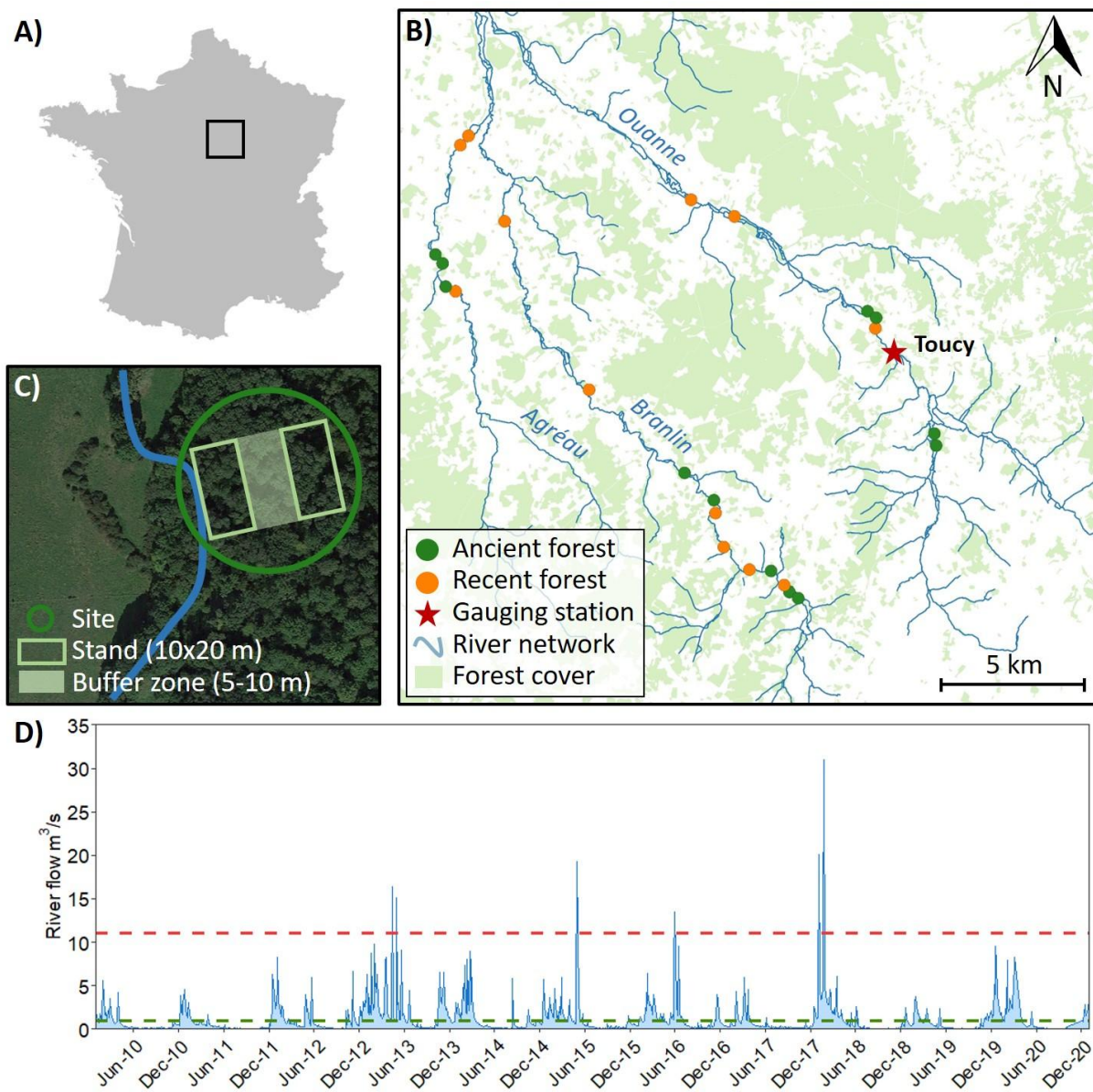
548

549 **Table 2.** Top-ranking species (n = 10) among 92 herbaceous species that explained most of the variation in species composition between ancient/recent forests and stands
550 far/close to the stream (Test = univariate test statistics from the multivariate GLM; p = p-values adjusted). To facilitate interpretation, species frequency in either
551 ancient/recent forests or far/close stands is provided (* = ancient forest indicator species).

Taxa	Forest continuity		Ancient	Recent	Taxa	Distance to stream		Far	Close
	Test	p				Test	p		
<i>Angelica sylvestris</i>	14.736	0.015	4.2%	29.2%	<i>Veronica montana</i> *	13.801	0.019	10.4%	35.4%
<i>Filipendula ulmaria</i>	13.023	0.023	8.3%	33.3%	<i>Stellaria holostea</i> *	12.566	0.045	2.1%	22.9%
<i>Milium effusum</i> *	10.759	0.104	20.8%	2.1%	<i>Elymus caninus</i> *	11.740	0.053	12.5%	35.4%
<i>Carex sylvatica</i> *	9.200	0.153	22.9%	4.2%	<i>Phalaris arundinacea</i>	9.305	0.209	0.0%	12.5%
<i>Caltha palustris</i>	9.178	0.195	0.0%	12.5%	<i>Veronica chamaedrys</i>	9.161	0.245	2.1%	18.8%
<i>Carex elongata</i>	9.178	0.219	0.0%	12.5%	<i>Atriplex prostrata</i>	7.544	0.480	0.0%	10.4%
<i>Vinca minor</i> *	9.178	0.228	12.5%	0.0%	<i>Viola reichenbachiana</i> *	7.057	0.531	14.6%	33.3%
<i>Glechoma hederacea</i>	8.268	0.294	25.0%	43.8%	<i>Geum urbanum</i>	6.579	0.572	31.3%	45.8%
<i>Scrophularia auriculata</i>	5.965	0.596	2.1%	14.6%	<i>Alliaria petiolata</i>	6.261	0.684	18.8%	35.4%
<i>Urtica dioica</i>	5.842	0.768	22.9%	39.6%	<i>Myosoton aquaticum</i>	6.019	0.741	0.0%	8.3%

552

553 **Figure 1.** Location of the study area in France (A), distribution of sampled riparian forests along the Ouanne
 554 creek and its tributaries (B), form of the sampling design used to survey plant communities in relation to their
 555 distance to the stream (C) and flow regime variations over the last 10 years at the Toucy gauging station (D,



556 dashed green line = interannual mean flow; dashed red line = 2-year return flood).

557 **Figure 2.** Constrained canonical analysis of principal coordinates of riparian understory (A) and overstory (B)
 558 communities, for each studied sites along the Ouanne creek and its tributaries, France. To facilitate graphical
 559 interpretation, the centroids for the interaction between forest continuity and distance to the stream are
 560 provided. In addition, the centroids in principle coordinate space of the most influential (i.e. with the highest
 561 cumulative deviance) understory and overstory species are provided (* = ancient forest indicator species, see
 562 also Appendix S3 and S4).

