Tree variability limits the detection of nutrient treatment effects on sap flux density in a northern hardwood forest

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The influence of nutrient availability on transpiration is not well understood, in spite of the importance of transpiration to forest water budgets. Soil nutrients have the potential to affect tree water use through indirect effects on leaf area or stomatal conductance. For example, following addition of calcium silicate to a watershed at Hubbard Brook, in New Hampshire, streamflow was reduced for 3 years, which was attributed to a 25% increase in evapotranspiration associated with increased foliar production. The first objective of this study was to quantify the effect of nutrient availability on sap flux density in a nitrogen, phosphorus, and calcium addition experiment in New Hampshire in which tree diameter growth, foliar chemistry, and soil nutrient availability had responded to treatments. We measured sap flux density in American beech (*Fagus grandifolia,* Ehr.), red maple (*Acer rubrum* L.), sugar maple (*Acer saccharum* Marsh.), white birch (*Betula papyrifera* Marsh.), or yellow birch *(Betula alleghaniensis* Britton.) trees, over five years of experiments in five stands distributed across three sites. In 2018, 3 years after a calcium silicate addition, sap flux density averaged 36% higher in trees in the treatment than the control plot, but this effect was not very significant (p= 0.07). Our second objective was to determine whether this failure to detect effects with greater statistical confidence was due to small effect sizes or high variability among trees. We found that tree-to-tree variability was high, with coefficients of variation averaging 39% within treatment plots. Depending on the species and year of the study, the minimum difference in sap flux density detectable with our observed variability ranged from 46% to 352%, for a simple ANOVA. We analyzed other studies reported in the literature that compared tree water use among species or treatments and found detectable differences ranging from 16% to 78%. Future sap flux density studies could benefit from power analyses to guide sampling intensity. Including pretreatment data, in the case of manipulative studies, would also increase statistical

power.

1 **Introduction**

2   Transpiration is affected by vegetation type and coverage as well as environmental

3 variables such as soil water availability and relative humidity (Aber & Federer, 1992; Foley et

4 al., 2000; Kite, 1998). Deciduous and evergreen forest types differ in annual water use due to the

5 length of the growing season (Daley et al., 2007) and tree species differ in sensitivity to drought

6 stress (Coble et al., 2017; Gu et al. 2015). In addition, the availability of nitrogen (N),

7 phosphorus (P), calcium (Ca), and silica may regulate transpiration rates (Cramer et al., 2008;

8 Ma, 2004; Matimati et al., 2014), due to the role of these elements in stomatal opening

9 (Laanemets et al., 2013; Lautner et al., 2007), leaf size (Wilkinson et al., 2007), canopy

10 reflectance (Sullivan et al., 2013) and root growth (Wright et al., 2011; Wurzburger and Wright,

11 2015).

12   Northern hardwood forests are poorly adapted to water stress due to the abundance of

13 water in this region (Pederson et al., 2014). Models predict that the northeastern USA will

14 experience more severe dry periods in the context of overall wetter conditions in the near future

15 due to climate change (Hayhoe et al., 2008). Other studies have suggested that water limitation

16 could become more important with intensifying summer droughts (Brzostek et al., 2014).

17 Because of these projections it is increasingly important to understand the controls on water use

18 in northern hardwood species. The effects of nutrient availability on water use are not currently

19 represented in forest hydrology models because they are poorly understood.

20   There have been conflicting reports on the effects of nutrient availability on tree water

21 use. An increase in sap flux density has been observed with the addition of multiple element

22 fertilizers in Norway spruce in northern Sweden (Phillips et al., 2001) and in eucalyptus forests

23   in Hawaii (Hubbard et al., 2004). Nitrogen addition reduced evapotranspiration rates in loblolly

24   pine in North Carolina (Ward et al., 2018). Calcium additions (lime and gypsum) increased

25   transpiration in central Amazonia (da Silva et al., 2008) and may have increased sap flow at

26   Hubbard Brook in New Hampshire, based on an observation of decreased stream flow for three

27   years after a calcium silicate addition before returning to pretreatment rates (Green et al., 2013).

28   In 13 stands distributed across three sites in the White Mountains of New Hampshire, N

29   and P have been added in full factorial combination since 2011 to study Multiple Element

30   Limitation in Northern Hardwood Ecosystems (MELNHE). By 2013, foliar nutrients had

31   responded to treatment (Wild and Yanai, 2015). Relative basal area growth across all 13 stands

32   responded to P addition but not to N addition by 2015, based on the average tree (Goswami et

33   al., 2018), but the dominant trees grew more in response to N addition (Hong et al., 2022). The

34   MELNHE study design included calcium silicate additions in seven of the stands to test the

35   hypothesis that an increase in tree water use explained the reduction in runoff following the

36   earlier calcium silicate addition at Hubbard Brook (Green et al., 2013).

37   One goal of this study was to determine the role of water use by trees in reducing runoff

38   after a calcium silicate addition by measuring sap flux density of five common northern

39   hardwood species in plots treated with calcium silicate at the same rate as the whole-watershed

40   addition at Hubbard Brook (Green et al. 2013). We also investigated the importance of other

41   nutrients to tree water use by quantifying sap flux density in plots receiving additions of N and P.

42   We predicted that sap flux density would increase with the addition of a limiting nutrient,

43   because nutrient availability is a driver of productivity and photosynthesis. Additionally, we

44   calculated minimum detectable differences in this study and in previously published studies to

45   determine whether failures to detect treatment effects were due to small effect sizes or to high

46 variability among trees. Power analyses of studies such as these can help to guide future research

47 plans.

Methods

48 **Methods**

49 **Site Description**

50   We studied tree water use in five naturally regenerated hardwood stands located in three

51 forested sites in the White Mountain National Forest, New Hampshire, USA: two in each of the

52 Bartlett Experimental Forest (44º 02' N, 71° 17' W) and Hubbard Brook Experimental Forest

53 (43º 93' N, 71° 73' W), and one in Jeffers Brook (44º 03' N, 71° 88' W; Table 1). The climate is

54 humid continental, with a mean annual temperature of 4.4 ºC and precipitation of 1400 mm

55 (Bailey et al., 2003; Smith & Martin, 2001). Soils are moderately well to well drained (Schaetzl

56 et al., 2009), coarse-loamy Spodosols and Inceptisols developed in glacial drift derived from

57 granitic and metamorphic silicate rocks (Vadeboncoeur et al., 2012; Vadeboncoeur et al., 2014).

58 Dominant tree species are American beech (*Fagus grandifolia* Ehrh.), sugar maple (*Acer*

59 *saccharum* Marsh.), and yellow birch *(Betula alleghaniensis* Britton) in mature stands with the

60 inclusion of white birch (*B. papyrifera* Marsh.) and red maple (*A. rubrum* L.) in successional

61 stands (Fatemi et al., 2012; Naples & Fisk, 2010). Tree height ranged from 9.9 m to 21.3 m in

62 the successional stands and 10.3 m to 33.2 m in the mature stands.

63   These five stands are part of a study of Multiple Element Limitation in Northern

64 Hardwood Ecosystems (Table 1; Hong et al., 2021). Within each of these stands there are four

65 plots measuring 50 x 50 m, except for Hubbard Brook Successional, which has 30 x 30 m plots.

66 These plots have been treated since 2011 with 30 kg ha^{-1} yr^{-1} of N as NH_4NO_3, 10 kg ha^{-1} yr^{-1} of

67 phosphorus as NaH_2PO_3, both N and P, or were left untreated. A fifth plot in these stands

68   received a one-time application of 1150 ha^{-1} of calcium silicate (wollastonite) in 2011, or in 2015

69   for Hubbard Brook Successional.

Field Methods

71   Sap flux density measurements were taken during the summers of 2013, 2014, 2015,

72   2017, and 2018 for periods of 7 to 48 days. In 2013 and 2014, we examined three tree species in

73   three mature stands in plots that received calcium silicate and control treatments (6 plots total;

74   Table 2). In 2015, our goal was to assess the effects of N and P treatments (4 plots) on one

75   species in one successional stand (Table 3). During those years, data were sparse due to poor

76   contact between the probes and the sapwood, but this problem was corrected in 2017. In 2017,

77   one species in a mature stand in Bartlett was examined, and in 2018, three species were

78   examined in the Hubbard Brook Successional stand. The number of trees of each species

79   monitored in each plot varied by stand and year (Tables 2 and 3).

80   Sap flux density measurements were made using the thermal dissipation method (Granier,

81   1987). This technique used a pair of stainless-steel probes 20 mm long and 1.8 mm in diameter.

82   One probe was wrapped with copper constantan thermocouple wire (Type T) and generated a

83   constant flow of heat (0.2 W). The other was a reference probe that received no heat. Both

84   probes were coated with heat-conducting paste and inserted into aluminum sleeves 20 mm long

85   and 2.4 mm in diameter for protection during insertion into the tree. The thermal dissipation

86   method may underestimate sap flux density depending on species, portion of probe in the

87   heartwood, and wood type (Flo et al., 2019; Peters et al., 2018), but a consistent bias would not

88   impair our ability to detect treatment effects. We tested for treatment differences in sap flux in

89   the youngest xylem; reporting sap flow per tree or scaling to the stand level would require

90   information on sapwood depth, which was not measured.

91   We selected trees with full canopies, without noticeable dead branches or cankers. A total

92   of 202 trees were instrumented, of which 174 produced usable data. The number of trees

93   instrumented per species per plot ranged from 1 to 6, averaging 3.2 ± 1.1 (standard deviation;

94   Tables 2 and 3). Before probe installation, $\sim 4~cm^2$ of bark was removed to the cambium at a

95   height of 1.37 m for the reference probe and 1.47 m for the heated probe on the south-facing side

96   of the tree. After the bark was removed, a hole 21 mm deep and 2.8 mm in diameter was drilled

97   in the middle of the exposed cambium for each probe.

98   Once probes were inserted, they were protected from precipitation with a plastic cover

99   sealed with acid-free silicone caulk. Closed-cell reflective polyethylene insulation was stapled

100   over the plastic cover to shield solar radiation. The probes were connected to cables no longer

101   than 20 m to minimize loss of power from cable resistance, trampling by humans, and

102   disturbance by wildlife.

103   Temperature differences between the heated and reference probes in each plot were

104   recorded every 15 minutes as an average of thirty 30-second readings on a multiplexor and

105   stored on a multichannel data logger (Campbell Scientific CR800). To power the data logger and

106   multiplexer, the first study, in 2013, used two 12 V deep-cycle marine batteries per plot charged

107   by two solar panels; later studies used three batteries linked together.

108   Gaps in usable data were usually due to cable disturbances or improper probe installation;

109   in the first year solar panels proved insufficient to maintain their charge, and data were very

110   spotty. The number of days of usable data was also improved by more frequent maintenance of

111   wire connections, probes, and batteries.

112   **Data Processing**

113   Temperature differences were converted into sap flux density using BaseLiner (version

114   3.0.10, developed by Ram Oren, Duke University; Oishi et al., 2016). BaseLiner used the

115   following equation to calculate sap flux density (g H_2O m^{-2} of sapwood day^{-1}):

116    $$Sap\ Flux\ Density = 119 \times \left(\frac{\Delta Tmax - \Delta T}{\Delta T}\right)^{1.23} \tag{1}$$

117   where the constants, 119 (Watts $°C^{-1}$) and 1.23, were derived using the quantity of heat applied to

118   the probes (Granier, 1985; Lu et al., 2004). ΔT_{max} (°C) is the "baseline" value that was manually

119   chosen as the maximum temperature difference between the two probes from 10:00 PM to 4:30

120   AM EDT. ΔT_{max} was set for each day for each tree, defining nocturnal flux as zero, which

121   accounted for any thermal drift that may have occurred from day to day. ΔT (°C) is the

122   temperature difference between the probes at each 15-minute observation period. We used the

123   same equation for all trees because species-specific coefficients were available for only one of

124   the five species studied (Peters et al., 2021).

125   The experimental unit was a treatment plot, and the observational unit was a tree.

126   Because of the many barriers to continuous collection of high-quality data, selecting data for

127   analysis was an important step. Twenty-eight trees (not shown in Tables 2 and 3) never produced

128   usable data due to faulty probe installation in the early years of the study. For each tree, we used

129   the average daily sap flux density calculated from summed 15-minute averages for each day.

130   Days used in analyses were mostly dry and sunny (PAR > 800 W/m^2 for at least seven hours).

131   Days were excluded from analyses if sap flux values did not follow the characteristic diurnal

132   curve or if the majority of the probes in the stand were not functioning simultaneously (Tables 2

133   and 3).

134   **Data Analysis**

135   The five studies of calcium silicate addition were analyzed in ANOVA Type III sum of

136   squares with average daily sap flux density as the response variable and treatment, species, and

137   the number of years post treatment as categorical fixed effects. The post-treatment years were

138   nested within two categories: "early" and "late," based on the finding that evapotranspiration

139   increased for 3 years after a calcium silicate addition and then returned to normal (Green et al.,

140   2013). Treatments were nested within stands and stands were treated as a random effect. The

141   assumption of normality of residuals was met through a logarithmic transformation of sap flux

142   density. We tested a model that included additional interaction terms, but the Akaike's

143   Information Criterion (AIC), 44.4, was higher than that of the simpler model (1.48) (Table S1).

144   Data from 2018 were analyzed independently using the same model without time since

145   treatment. For this analysis, the assumption of normality of residuals was met without

146   transformation.

147   Our study design included all combinations of N and P addition, but no combinations of

148   calcium with N or P. To take advantage of the factorial N x P addition, we analyzed the N and P

149   treatments separately from the calcium silicate addition treatments. Thus, the three studies in N

150   and P addition plots were analyzed together in a randomized full factorial ANOVA with average

151   daily sap flux density as the response variable and species and stand as fixed effects. Three

152   stands were studied in three different years, which precluded distinguishing the effects of stands

153   from the effects of the year of measurement. A model including the interaction of species and

154   treatment was not an improvement, according to the AIC (97 compared to 86) (Table S2).

155   Normality of the residuals was achieved by logarithmic transformation of sap flux density.

156   Additionally, the year with the best data quality (2018) was analyzed independently using the

157   same model with the exception of excluding stand as a fixed effect. For this analysis, the

158   assumption of normality of residuals was met without transformation. We tested whether nutrient

159   additions (Ca and N x P) affected sap flux density at any time of day, using trees from 2017,

160   which had the largest sample size of a single species (sugar maple). These effects were tested for

161   each hour over three days using ratios of instantaneous sap flux density to the average for the

162   day. There were no significant treatment effects when a Bonferroni correction was applied ($p \geq$

163   0.32). Therefore, only results using daily average sap flux densities were reported.

164   All analyses were conducted using the "lme4" package in *R* version 3.5.2 (R

165   Development Core Team 2018).

166   Our second objective was approached by calculating coefficients of variation (CV) and

167   minimum detectable differences for our study and published studies. Coefficients of variation

168   across trees were calculated for each treatment by stand and year studied using the average daily

169   sap flux density per tree. Coefficients of variation across treatments were also calculated for each

170   stand and year studied for comparison to within-treatment CV's. Published studies were chosen

171   for minimum detectable difference analyses from a search using the keywords "sap flux," "sap

172   flow," "trees", and "treatment addition," and were selected if they reported sample sizes, mean

173   sap flux density, and standard deviation or error for each treatment or species of interest.

174   The minimum detectable difference in sap flux density among treatments or species in

175   our study and for published studies was calculated using the following equation (Zar, 1984):

176                 $$Minimum\ Detectable\ Difference = \sqrt{\frac{2kS^2\phi^2}{n}} \hspace{2cm} (2)$$

177   where k is the number of treatments, S^2 is the sample variance, ϕ^2 is the non-centrality parameter

178   dependent on β and α, and n is the sample size. Power ($1-\beta$) was set at 0.8 and α at 0.05. For an

179   ANOVA, S^2 is the mean squared error. This equation was rearranged to calculate the sample size

180   needed to detect a 20% and 50% difference using data from 2017 and 2018. To estimate the

181   minimum detectable difference from published studies, S^2 was the average of the variances

182   across treatments (Eq. 2).

183   **Results**

184   **Characteristics of Sap Flux Density**

185   Sap flux density peaked between 12:30 and 3:00 PM and was lowest between 2:30 and

186   4:30 AM Eastern Daylight Savings Time. Trees varied consistently in sap flux density across

187   days (Figure 1).

188   The first year, 2013, had the highest CV's among the five studies (Table 4). Coefficients

189   of variation across trees within plots for each year ranged from 9% to 136% and averaged 38%

190   within treatment plots across trees. CV's across treatments averaged 17% with a range of 8% in

191   Bartlett Mature in 2017 to 49% in Jeffers Brook Mature in 2013. Average CV's within treatment

192   plots were higher than CV's across treatments.

193   **Effects of Nutrient Additions**

194   In 2018, the year with the best data quality, the effect of Ca addition was a marginally

195   significant increase of 36% ($p=0.07$). With all years included, the effect of calcium silicate

196   addition on sap flux density was not consistent ($p= 0.30$ for the main effect of treatment). Sap

197   flux density did not differ consistently between "early" (2- and 3-years post treatment) and "late"

198   (4 and 6 years post treatment) years ($p=0.26$) nor was there a difference in response to a calcium

199   silicate addition as the number of years post treatment increased (p=0.88 for the interaction of

200   treatment and time period). The five species were indistinguishable in sap flux density ($p = 0.42$;

201   Figure 2; Table 5).

202   The effects of N and P on sap flux density were studied in white birch in Bartlett

203   Successional in 2015, sugar maple in Bartlett Mature in 2017, and red maple, sugar maple, and

204   white birch in Hubbard Brook Successional in 2018 (Table 3). Sap flux density differed across

205   these three studies ($p <0.01$), which could be because sites were different or because conditions

206   differed during the measurement periods among the three years. Sap flux density in the Hubbard

207   Brook Successional stand, measured in 2018 after 7 years of fertilization, was 63% higher than

208   in Bartlett Successional measured in 2015 and Bartlett Mature measured in 2017. There was,

209   however, no detectable difference in sap flux density due to the main effects of N ($p= 0.50$) or P

210   ($p= 0.95$) or their interaction ($p= 0.33$). Species were also indistinguishable in sap flux density

211   ($p= 0.58$; Figures 3 and 4; Table 6).

212   In 2018, the best year for data quality, there was still not a consistent effect of N ($p=$

213   0.26) or P ($p= 0.10$) addition on sap flux density. Trees in the N and P plots had 14 and 20%

214   higher sap flux densities, respectively, than those in the controls but trees in the NP plot did not,

215   resulting in a weak N x P treatment interaction ($p= 0.06$). This is apparent in Figures 3 and 4,

216   where the filled blue symbols, showing the plots receiving the element not depicted on the x and

217   y axes, fall below the open symbols. However, the interaction is based on only one plot receiving

218   both treatments and is thus not very convincing: in this case, an environmental factor that

219   differentially affected water use in one treatment plot would explain the observed "interaction"

220   of N and P, whereby the NP plot had lower values than predicted by the main effects of N and P,

221   which were positive, if not significant. The p value of 0.10 for the main effect of P seems

222 possibly worthy of attention, but both of the two main effects became less significant when the

223 interaction term was excluded from the model ($p = 0.66$ for P), again showing that the analysis

224 based on only one stand is not very robust.

225 **Power Analyses**

226 The lack of significant treatment effects does not necessarily mean that effect sizes were

227 small (Amrhein et al., 2019); rather, the minimum detectable differences for a simple ANOVA

228 were large, ranging from 50 to 1582 g m^{-2} day^{-1} (50% to 352% of the mean) for Ca additions and

229 from 779 to 1209 g m^{-2} day^{-1} (46% to 134%) for N x P additions, depending on the year (Figure

230 5). Improvements in methods and larger sample sizes resulted in smaller detectable differences

231 over time, associated with lower variability from tree to tree (Table 4). Even in 2018, where

232 detectable differences were smallest, the variation from tree to tree within a plot was large

233 (Figure 6).

234 The average number of trees monitored per plot increased over time from 2 in 2013 to 9

235 in 2018. Even with this increase in sample size, we were still unable to detect a treatment effect.

236 We calculated the number of trees needed to detect a given effect size, using the studies with the

237 best data quality, and assuming there were no differences among species. To detect a 20%

238 treatment effect on sap flux with 95% confidence would have required 119 trees in each

239 treatment plot in 2017 or 48 trees in each plot in 2018. The number of trees needed to detect a

240 50% difference would be 19 for 2017 and 9 for 2018, given the variance in sap flux density we

241 measured in those years.

242 **Minimum Detectable Differences for Published Studies**

243   Effect sizes reported in eight previously published studies of tree water use ranged from 8

244   to 243% (Table 7). The largest reported effect size was a 90% increase in sap flux density with

245   the addition of N (80 kg ha^{-1} yr^{-1}) in loblolly pine (*Pinus taeda*) seedlings (Samuelson et al.,

246   2008). A 43% increase was reported with the addition of macro and micronutrients to a

247   *Eucalyptus saligna* plantation in Hawaii (Hubbard et al., 2004), and a 35% increase was detected

248   in a study involving the addition of P (50 kg ha^{-1} of P$_2$O$_5$$^{-1}$) and Ca (2 t ha^{-1} of CaCO$_3$) on *Vismia*

249   *japurensis, Bellucia grossularioides,* and *Laetia procera* in Amazonia (da Silva et al., 2008).

250   Minimum detectable differences ranged from 16 to 75% in temperate forests and 52 to

251   74% in tropical and subtropical forests (Table 7). Two studies of temperate deciduous forest

252   species had lower calculated detectable differences than ours. A study of eight tree species in

253   four forest types in Wisconsin had a detectable difference in sap flux density of only 16% with a

254   sample size of 8 per species, due to low variability among trees (Ewers et al., 2002). Another

255   study in the same region used more trees from a larger area, resulting in higher variability and

256   slightly higher detectable differences (Loranty et al., 2008). Some studies had much higher effect

257   sizes than ours (Kunert et al., 2010; Nagler et al., 2003; Samuelson et al., 2008) and thus were

258   able to detect them with statistical confidence although their power to detect a difference was not

259   better. Publication of insignificant findings is rare (Møller and Jennions, 2001); one earlier study

260   took place in two MELNHE stands (including one that we studied, Bartlett Mature) with effect

261   sizes well below detection, similar to ours (Hernandez-Hernandez, 2014).

262   **Discussion**

263   The initial impetus for this study was the surprising decrease in stream flow for three

264   years following a calcium silicate addition at Hubbard Brook, where tree water use was invoked

265 as a likely mechanism (Green et al., 2013). Unfortunately, even in 2018, the year with the lowest

266 tree-to-tree variability and the highest replication, our statistical power was too low to detect a

267 response of sap flux density to calcium addition smaller than 50% with an α of 0.05 (Figure 5);

268 we obtained a p value of 0.07 for an average increase in sap flux density of 36%, which is larger

269 than the 25% increase in water use postulated by Green et al. (2013). Thus, we lack the statistical

270 confidence to either support or contradict the explanation that increased sap flux density

271 accounted for the decline in runoff in the earlier calcium addition experiment.

272 There are other possible mechanisms for increased water use in response to nutrient

273 addition. In the MELNHE study, the relative basal area increment of the average tree increased

274 in response to P addition (Goswami et al., 2017), and larger trees grew more in response to N

275 addition (Hong et al., 2022). In the calcium silicate addition, trees grew more than in the

276 unfertilized control (Battles et al., 2014; Huggett et al., 2007). An increase in diameter growth

277 could result in increased sapwood area (Nilsson et al., 2021), which could conduct water to a

278 greater leaf area (Wang et al., 2012) without an increase in sap flux density (Hubbard et al.,

279 2004). It is also possible that nutrient availability affects the length of the growing season

280 (Escudero et al., 1991), which would affect annual water use without affecting sap flux density.

281 Other studies of sap flux density have reported statistically significant effects to treatment

282 additions, with effect sizes as low as 35% (Table 7). The difference detectable with our factorial

283 ANOVA comparing N and P additions is somewhat smaller than the 46% shown in Figure 5,

284 because Equation 1 describes power for a simple ANOVA. The benefit of factorial designs is

285 evidenced by two of the published studies we analyzed. The study involving combinations of

286 phosphate, lime, and gypsum additions (da Silva et al., 2008) was able to detect a significant

287 difference of 35%, whereas our calculated minimum detectable difference for a simple ANOVA

288   was 52%. Similarly, the full factorial study of irrigation and N fertilization (Samuelson et al.,

289   2008) reported a 90% increase with N fertilization with a p-value of 0.01, which shows greater

290   power than the detectable difference of 97% calculated using Equation 2 for a simple ANOVA

291   with α = 0.05. Unfortunately, without a power analysis for factorial designs, we cannot better

292   quantify the effect size ruled out by our findings in the N x P addition experiment. It remains

293   possible that nutrient availability has an ecologically important effect on sap flux.

294   Our study might have benefitted from pretreatment measurements since sap flux density

295   varied consistently from tree to tree (Figures 1 and 6). Pretreatment data, like factorial designs,

296   can confer additional power. The only study we analyzed that collected pretreatment data

297   (Hubbard et al., 2004) reported an effect size of 43% with p = 0.04, which seems consistent with

298   the minimum detectable difference we calculated (40% with α = 0.05). Thus, it seems unlikely

299   that the pretreatment data were used in the statistical analysis.

300   In addition to including pre-treatment measurements, future sap flux density studies could

301   benefit from incorporating explanatory variables such as leaf area, canopy position, and stomatal

302   conductance, since these characteristics have important influences on sap flux density (Green et

303   al., 2013; Hubbard et al., 2004; Peters et al., 2021; Phillips et al., 2001). Environmental variables

304   such as soil moisture and light environment may also be of interest since they influence sap flux

305   density (Oren et al., 1998a; Oren et al., 1998b). Sapwood area measurements not only allow for

306   scaling up to whole-tree water use but also make it possible to ensure that probes are within the

307   sapwood. If probes are inserted into heartwood, which may have occurred in our study, estimated

308   sap flux measurements can be reduced up to 50% (Clearwater et al., 1999). Many of these

309   variables likely contributed to the variation we observed among trees.

Conclusion

310

311   This study aimed to assess the influence of soil nutrient availability on sap flux density in

312 northern hardwoods. Specifically, we sought to verify whether increased tree water use could

313 explain the observed reduction in runoff following a calcium silicate application in an earlier

314 study, and we tested for effects of N and P addition on sap flux density in a long-term N by P

315 factorial addition experiment. Unfortunately, poor statistical power due to high tree-to-tree

316 variability prevented detection of potentially important treatment effects. Previous studies that

317 reported significant differences had larger effect sizes; few had sufficient statistical power to

318 detect small differences. To improve the ability to detect a treatment effect, future studies should

319 instrument more trees to increase statistical power, collect pretreatment data, when applicable, to

320 control for tree-to-tree variability, and include additional tree metrics such as leaf and sapwood

321 areas.

Acknowledgements

322

323   Mark Green, Michele Pruyn, John Drake, and Pam Templer provided useful information

324 on the mechanisms of sap flux and what variables to consider when monitoring tree water use.

325 Matt Vadeboncoeur, Jamie Harrison, and Adam Wild helped in the design of this study by

326 explaining previous design flaws and their own recent findings. High school and undergraduate

327 students and summer interns helped build probes and instrument trees. Russell Briggs and Steve

328 Stehman provided statistical advice. The MELNHE project was funded by the USDA National

329 Institute of Food and Agriculture (2019-67019-29464) and by the National Science Foundation

330 (DEB-0949324) including via the Long-Term Ecological Research Network (DEB-1114804 and

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Figure 1

An example of sap flux density in nine trees continuously measured from July 19-21, 2018.

Each color represents one tree in the control plot in the Hubbard Brook Successional stand.

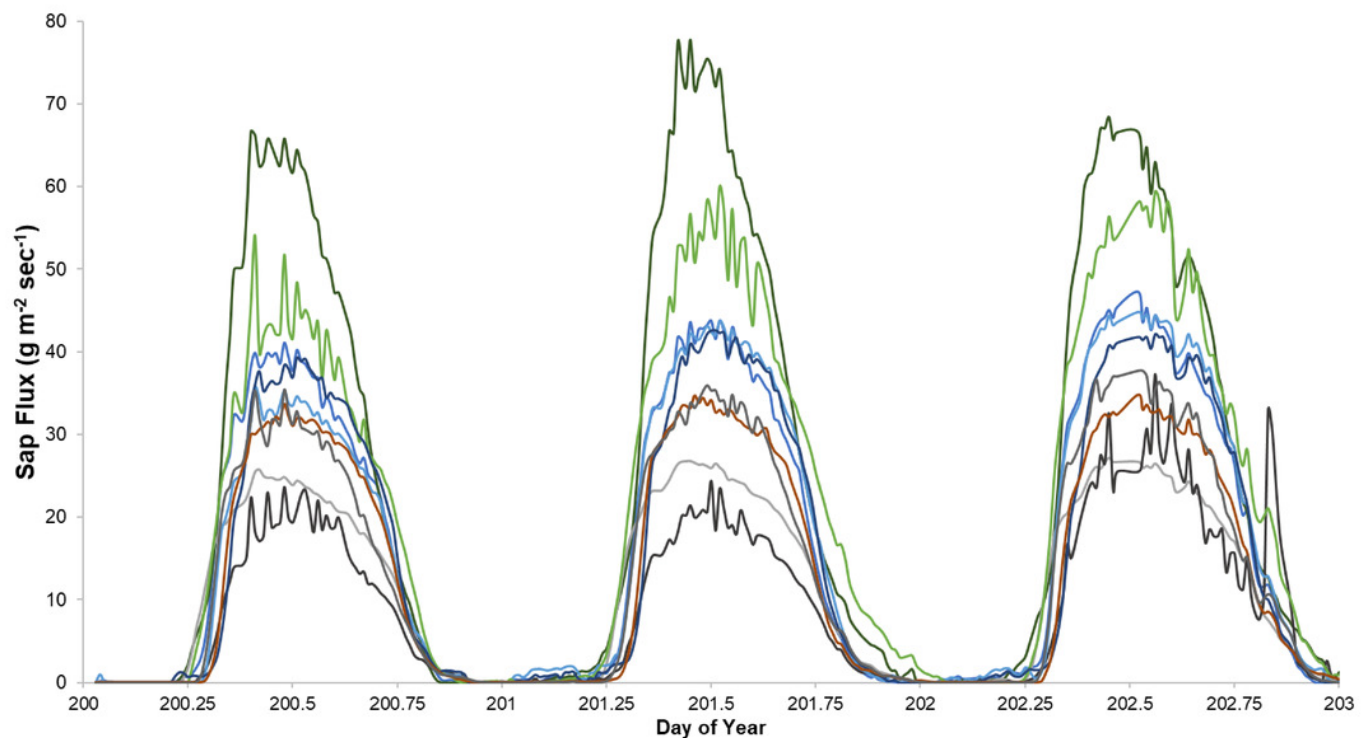



Figure 2

Response of sap flux to calcium silicate treatment.

Bars represent standard errors across trees. Symbols represent species and colors represent years with associated stands. The 1:1 line represents equal flow rates in control and Ca-treated plots.

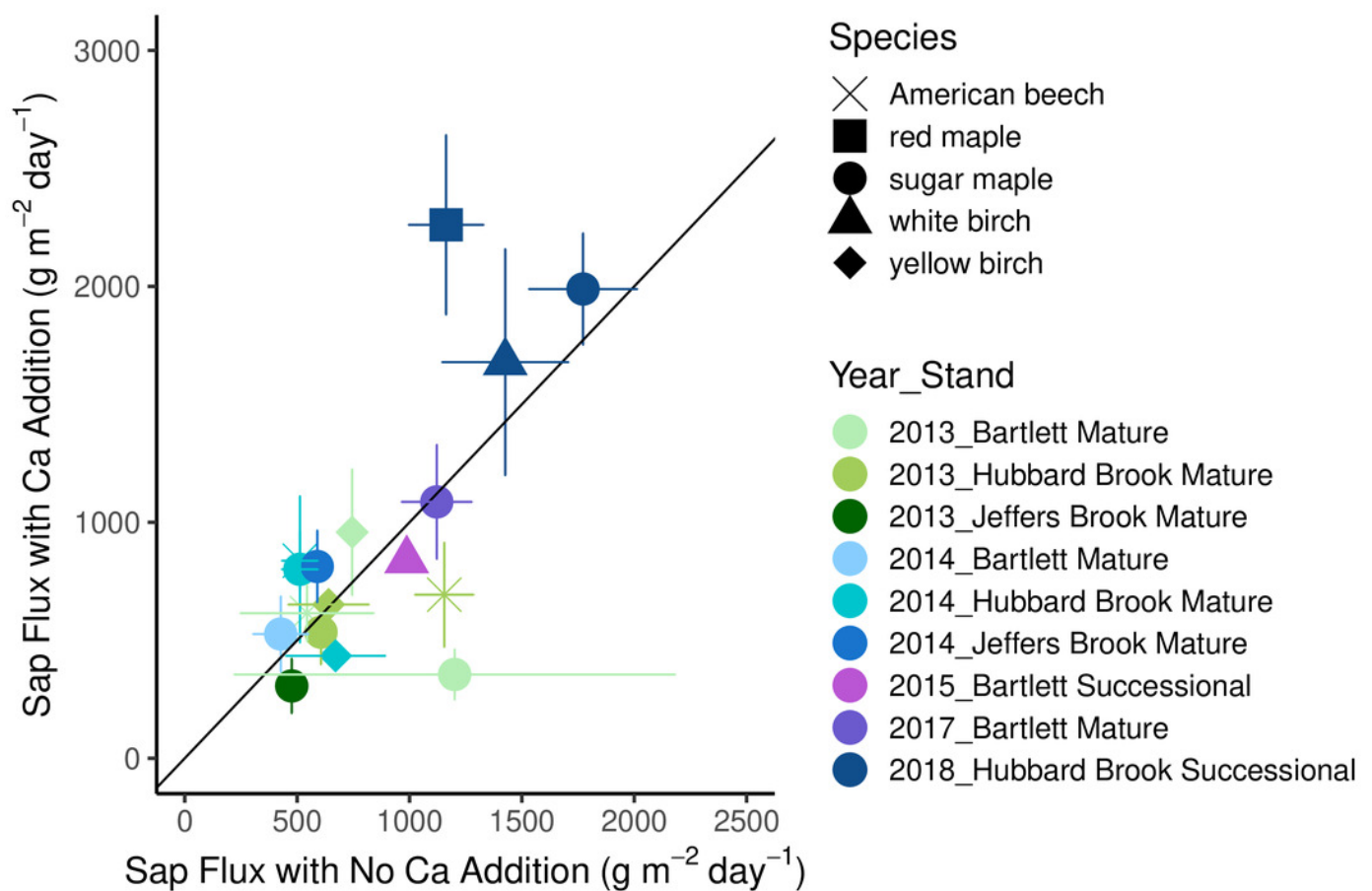



Figure 3

Responses of sap flux to nitrogen treatments.

Bars represent the standard error across trees. Species are represented by symbols; stands with the year studied are represented by colors. The 1:1 line represents equal sap flux rates in plots that received nitrogen additions (N and NP) and plots without nitrogen additions (Control and P).

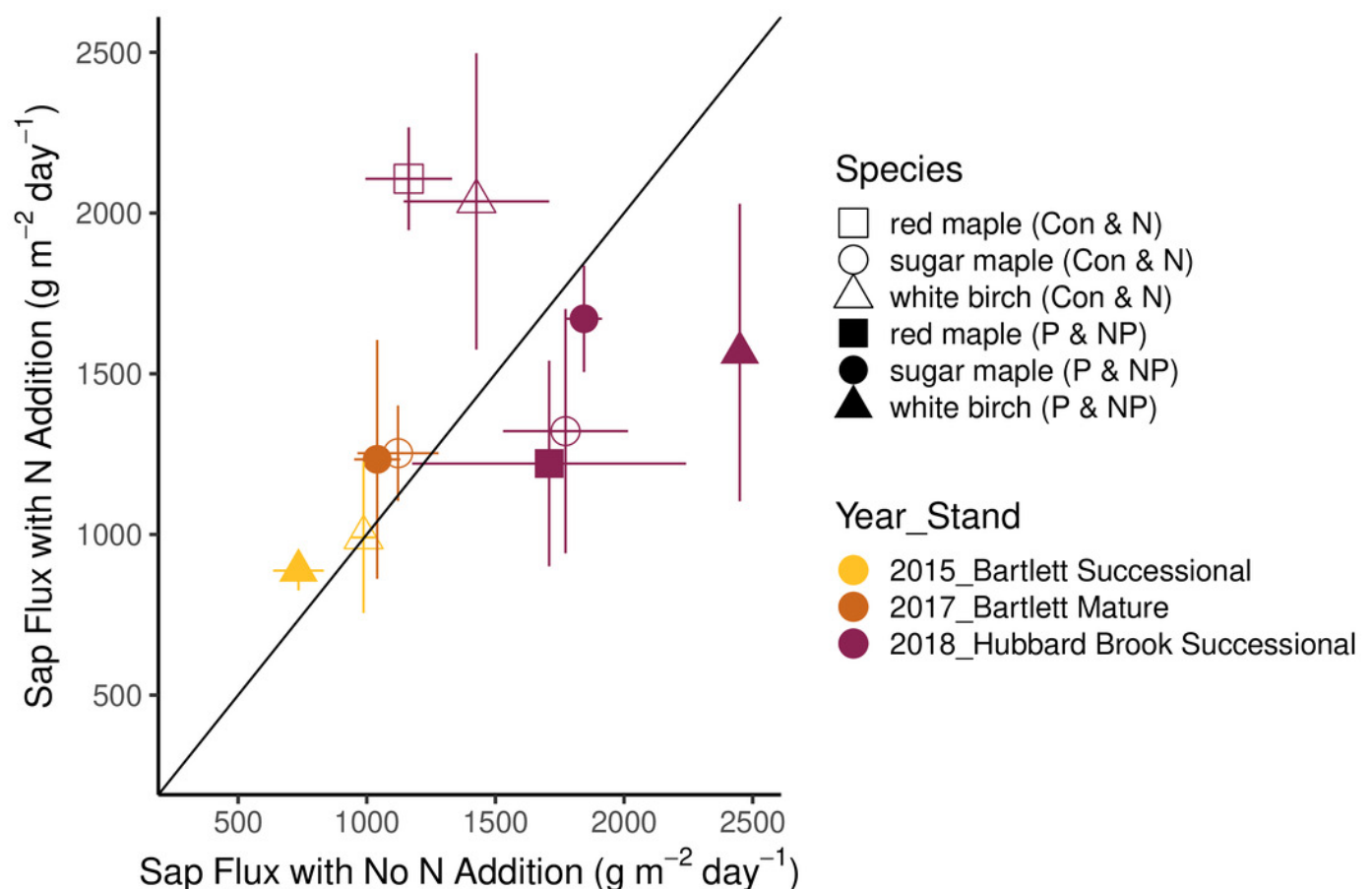



Figure 4

Responses of sap flux to phosphorus treatments.

Bars represent the standard error across trees. Species are represented by symbols; stands with the year studied are represented by colors. The 1:1 line represents equal sap flux rates in plots that received phosphorus additions (P and NP) and plots without phosphorus additions (Control and N).

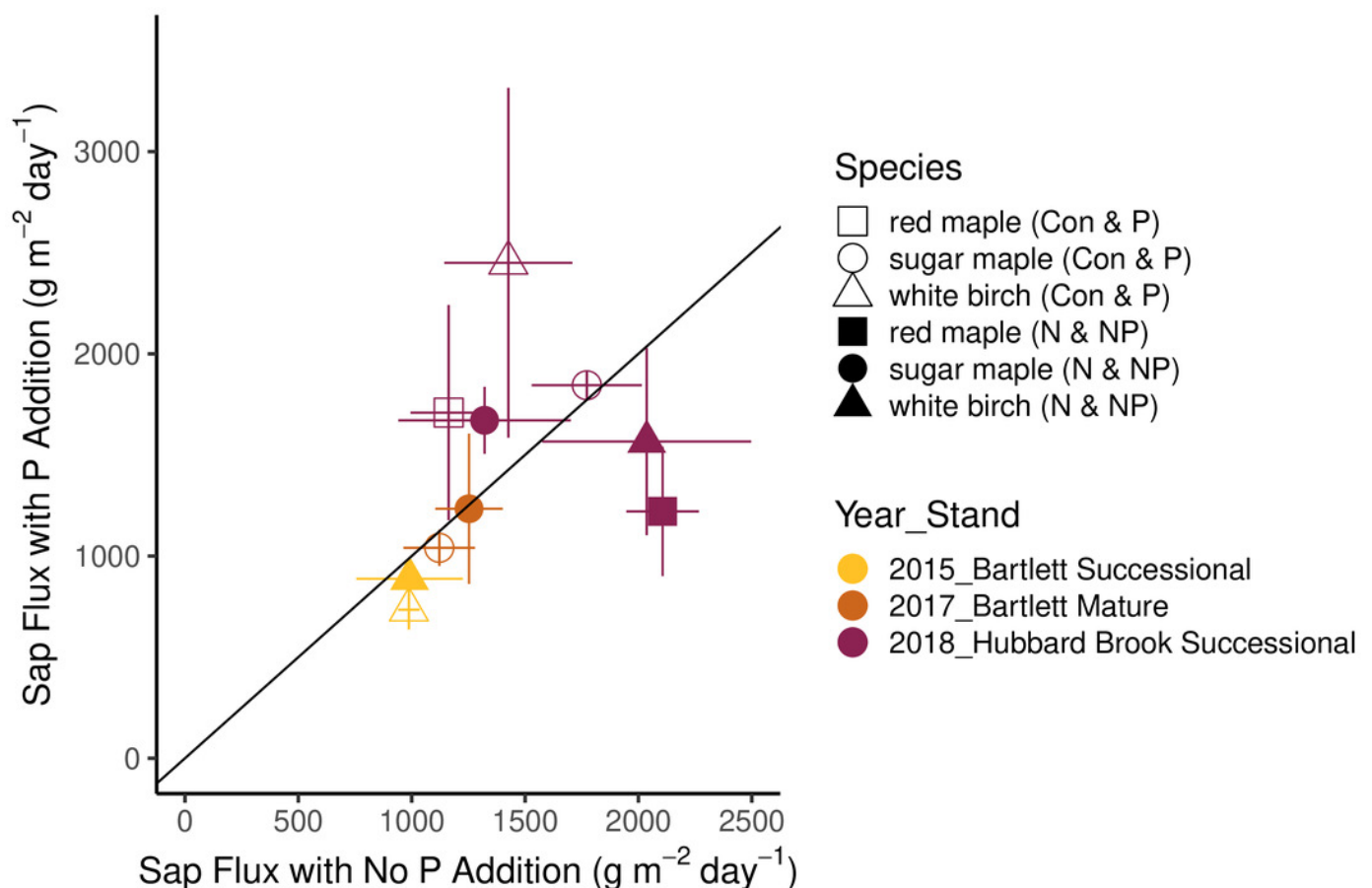



Figure 5

Minimum detectable treatment differences for each stand, based on a simple ANOVA.

Shapes represent stands and colors represent treatment comparisons. Control and calcium plots were studied from 2013 to 2018. Nitrogen and phosphorus additions were studied from 2015 to 2018. Detectable differences for the factorial N x P ANOVA are somewhat smaller than shown here.

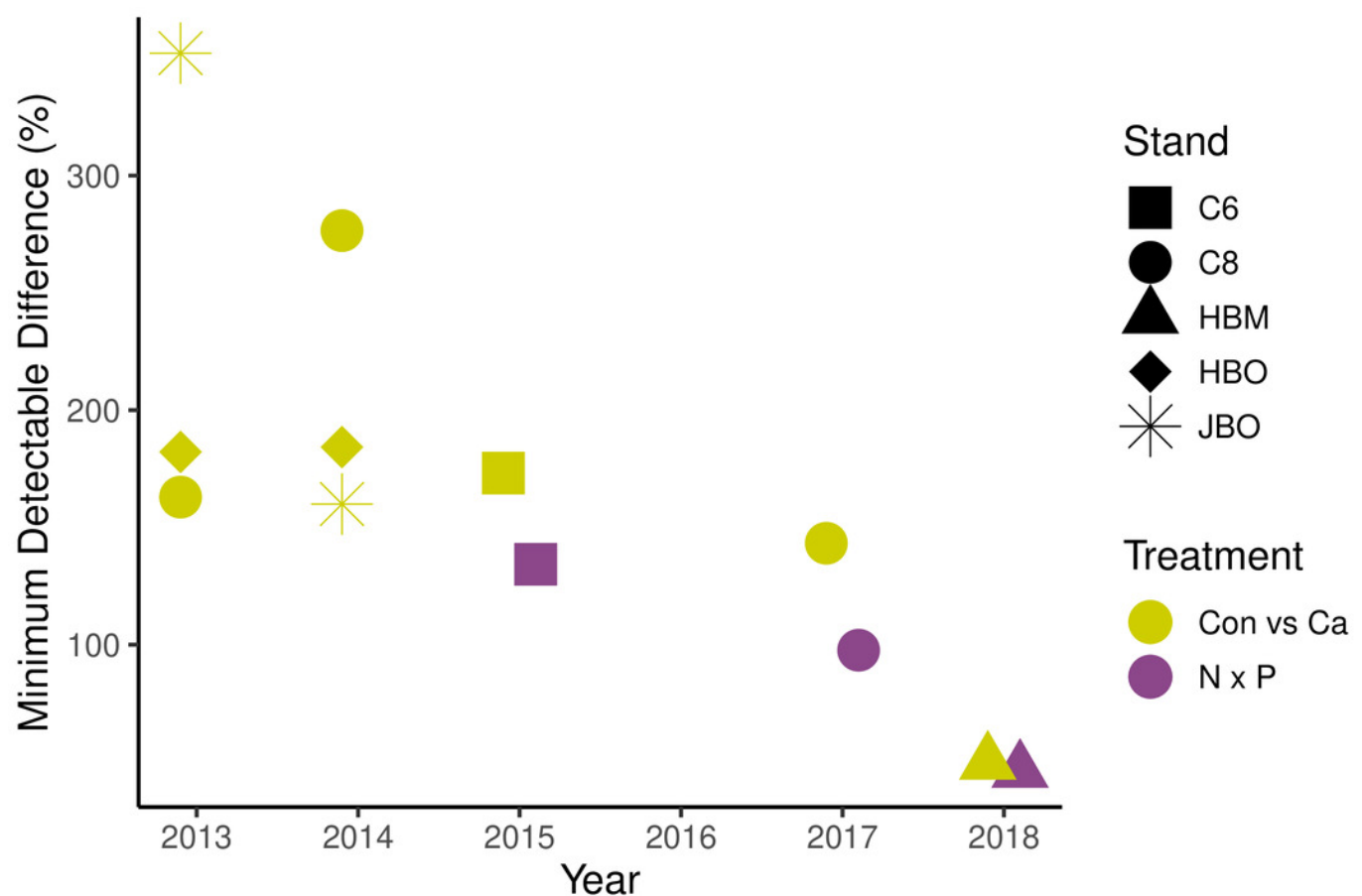
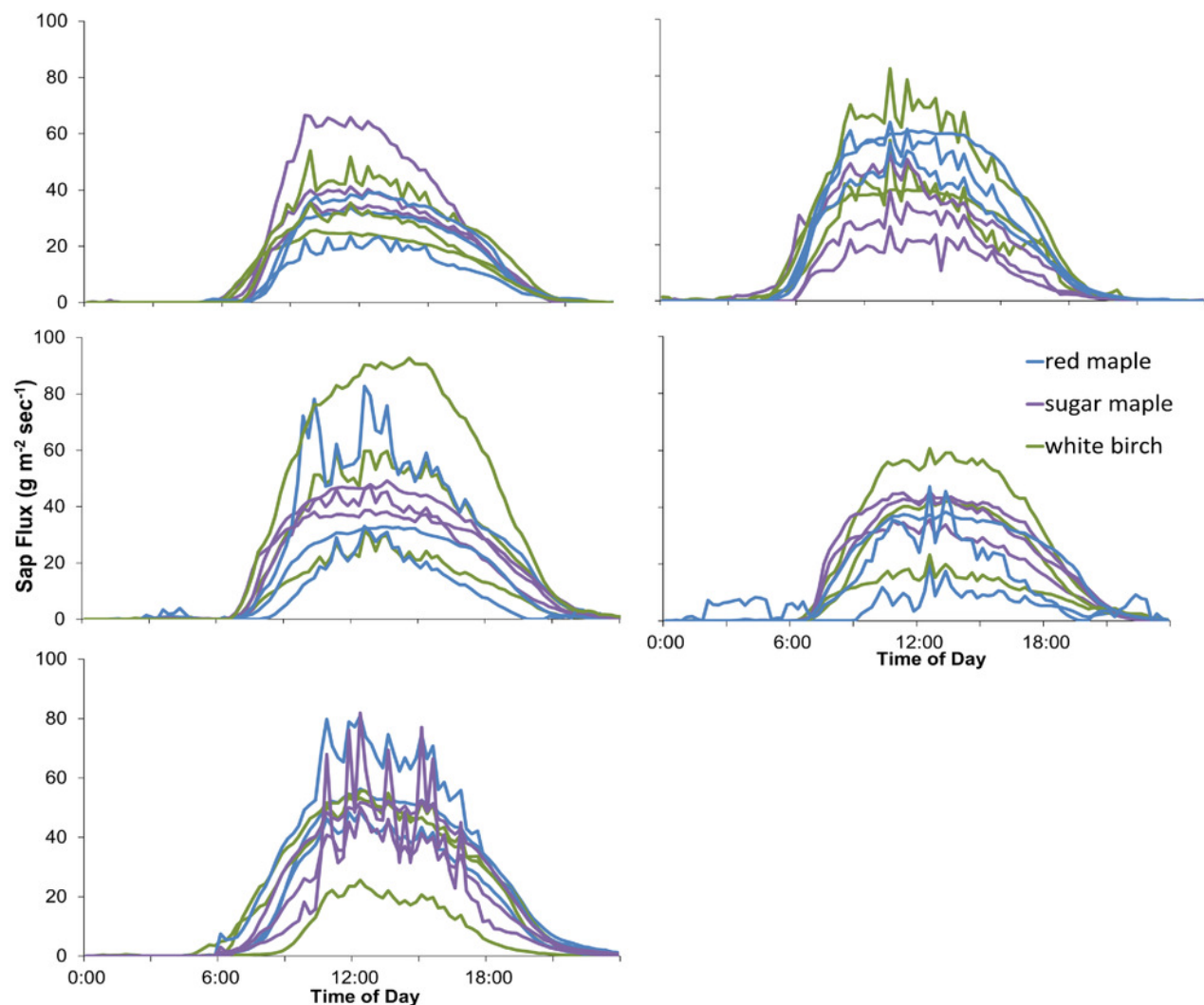



Figure 6

Diurnal sap flux density in the Hubbard Brook Successional stand on July 19, 2018.

Times are local daylight savings time. Each line represents an instrumented tree; there are 9 trees in each treatment colored by species.

Site descriptions of the five forested stands studied in the White Mountains of New Hampshire.

Date of stand initiation was determined from the last clearcut or heavy harvest event. Quadratic mean diameters and basal areas are based on trees with diameters greater than 2 cm. Stem density is calculated for trees greater than 10 cm in diameter.

Stand	Date of Stand Initiation	Elevation (m)	Slope (%)	Aspect	Quadratic Mean Diameter (cm)	Stem Density (count/ha)	Basal Area (m²/ha)
Bartlett Successional (C6)	1975	460	13–20	NNW	23.1	1487	29.5
Bartlett Mature (C8)	~1883	330	5–35	NE	41.2	589	41.0
Jeffers Brook Mature	~1900	730	30–40	WNW	35.4	680	35.9
Hubbard Brook Successional	1971	500	10–25	S	23.6	2749	15.4
Hubbard Brook Mature	~1910	500	25–35	S	36.5	538	29.5

Studies of sap flux density in response to calcium silicate addition showing the dates, number of years since treatment and the number of trees of each species used in analysis in each treatment plot.

The calcium silicate treatment was applied in 2011, except that in Hubbard Brook Successional it was applied in 2015.

Dates (# of Years Post Treatment)	Stand	Species	Number of Trees in Treatment	
			Control	Calcium Silicate
July 19, 2013 (2)	Jeffers Brook Mature	sugar maple yellow birch	4 3	2 1
August 5, 2013 (2)	Bartlett Mature (C8)	American beech sugar maple	2 3	3 3
August 5, 2013 (2)	Hubbard Brook Mature	American beech sugar maple yellow birch	2 2 2	2 2 1
June 22-24, 2014 (3)	Bartlett Mature (C8)	sugar maple	3	2
July 1, 2014 (3)	Hubbard Brook Mature	American beech sugar maple yellow birch	3 3 3	2 2 1
August 1, 2 and 5, 2014 (3)	Jeffers Brook Mature	sugar maple yellow birch	4 3	4 5
July 22 and 23, 2015 (4)	Bartlett Successional (C6)	white birch	4	4
July 31 and August 1, 2017 (6)	Bartlett Mature (C8)	sugar maple	5	4
July 19-21, 2018 (3)	Hubbard Brook Successional	red maple sugar maple white birch	3 3 3	3 3 3

Studies of sap flux density in response to nitrogen and phosphorus addition showing the date of the study, the number of annual nutrient applications prior to each study, and the number of trees of each species used in analysis in each treatment plot.

Dates (# of Years of Treatment)	Stand	Species	Number of Trees in Treatment			
			Control	Nitrogen	Phosphorus	Nitrogen + Phosphorus
August 1, 2015 (4)	Bartlett Successional (C6)	white birch	4	4	5	4
July 31 and August 1, 2017 (6)	Bartlett Mature (C8)	sugar maple	5	6	6	5
July 19- 21, 2018 (7)	Hubbard Brook Successional	red maple sugar maple white birch	3 3 3	3 3 3	3 3 3	3 3 3

Coefficient of variation (CV) of sap flux for each treatment plot in each study year.

CV's were calculated as the standard deviation of sap flux for all the trees in a treatment plot divided by the average for that plot.

Year	Stand	Treatment	CV (%)
2013	Bartlett Mature (C8)	Control	136
		Calcium	58
	Hubbard Brook Mature	Control	36
		Calcium	32
	Jeffers Brook Mature	Control	34
		Calcium	32
2014	Bartlett Mature (C8)	Control	36
		Calcium	34
	Hubbard Brook Mature	Control	40
		Calcium	45
	Jeffers Brook Mature	Control	16
		Calcium	35
2015	Bartlett Successional (C6)	Control	14
		Nitrogen	47
		Phosphorus	30
		Nitrogen + Phosphorus	14
		Calcium	9
2017	Bartlett Mature (C8)	Control	31
		Nitrogen	29
		Phosphorus	21
		Nitrogen + Phosphorus	67
		Calcium	44
2018	Hubbard Brook Successional	Control	30
		Nitrogen	36
		Phosphorus	47
		Nitrogen + Phosphorus	37
		Calcium	31

NONE

ANOVA table showing Type III sum of squares for calcium silicate addition studies.

The response variable is sap flux density, with calcium silicate treatment, species, and "Early" vs "Late" as categorical fixed effects. "Early" measurements were taken 2 or 3 years after the calcium silicate addition and "Late" were taken 4 or 6 years after the addition (Table 2).

	Df Numerator	Df Denominator	F Value	Pr(>F)
"Early" vs "Late"	1	333.77	1.2932	0.26
Treatment	1	6.14	1.2764	0.30
"Early" vs "Late x Treatment	1	8.45	0.0227	0.88
Species	4	20.27	1.0287	0.42

ANOVA table showing Type III sum of squares for N and P addition studies.

The response variable is average daily sap flux density and the listed explanatory variables are categorical fixed effects. The three stands were studied in three different years (Table 3).

	Sum of Squares	Df	F Value	Pr(>F)
N Treatment	0.071	1	0.460	0.50
P Treatment	0.001	1	0.004	0.95
NxP Interaction	0.146	1	0.944	0.33
Species	0.168	2	0.543	0.58
Stand	4.481	2	14.478	<0.001
Error	10.987	71		

Studies of sap flux density or sap flow among species or following nutrient additions in deciduous and evergreen trees, listed from lowest to highest minimum detectable difference.

Forest Type	Species or Treatment Studied (number of trees)	Effect Size and Significance	Minimum Detectable Difference (%)	Source
		Sap Flux Density		
Temperate Deciduous and Evergreen	*Pinus resinosa* (8) *Pinus banksiana* (8) *Acer saccharum* (8) *Populus tremuloides* (8) *Abies balsamea*(8) *Thuja occidentalis* (8) *Abies balsamea*(8) *Alnus regosa* (8)	Species was significant overall; pairwise comparisons ranged from 7.5 to 243% ($p<0.05$)	16	Ewers et al., 2002
Temperate Deciduous and Evergreen	*Alnus incana* (41) *Populus tremuloides* (79) *Thuja occidentalis* (9)	Not Reported	21–65	Loranty et al., 2008
Temperate Evergreen (*Eucalyptus saligna*)	Control (9) Micro and macronutrients (9)	43% increase with fertilization ($p=0.04$)	40	Hubbard et al., 2004
Temperate Deciduous	Control (9) Nitrogen (9) Phosphorus (9) Nitrogen + phosphorus (9) Calcium (9)	14% increase with N ($p= 0.26$); 20% increase with P (0.10) 36% increase with Ca treatment ($p= 0.07$)	46 (NxP) 50 (Ca)	This study (2018 only)
Tropical Evergreen (*Vismia japurensis, Bellucia grossularioides, Laetia procera*)	Control (9) phosphate (9) phosphate + lime (9) phosphate + lime + gypsum (9)	Phosphate + lime + gypsum treatment was 35% higher than phosphate + lime ($p<0.05$)	52	da Silva et al., 2008
Temperate Deciduous	*Quercus mongolica (5)* *Tilia amurensis (5)* *Ulmus davidiana (5)* *Cornus controversa (3)* *Acer mono (3)*	No Significance ($p=0.73$)	52–75	Jung et al., 2011

Tropical Deciduous	*Cedrela odorata* (4) *Anacardium excelsum* (4) *Hura crepitans* (4) *Luehea seemannii* (4) *Tabebuia rosea* (4) *Gmelina arborea* (4) *Tectona grandis* (4) *Acacia mangium* (4) *Terminalia amazonia* (4)	*A. excelsoum, L. seemannii* and *T. amazonia* were 113% higher than C. odorata and G. arborea ($p<0.05$)	61	Kunert et al., 2010
Temperate Deciduous	Control 7) Nitrogen (9) Phosphorus (9) Nitrogen + phosphorus (9)	12% increase with N and 12% increase with P ($p>0.05$)	66	Hernandez-Hernandez, 2014

Sap Flow Rate

Subtropical Evergreen (*Pinus taeda*)	Control (5) Irrigated(5) Nitrogen (5) Irrigated+Nitrogen (5)	90% increase with N fertilization ($p<0.01$)	74	Samuelson et al., 2008
Riparian Deciduous (Desert)	*Populus fremontii* (6) *Salix gooddingii* (6) *Tamarix ramosissima* (6)	*T. ramosissima* was 122% higher than *P. fremontii* and *S. gooddingii* ($p<0.05$)	78	Nagler et al., 2003