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ORIGINAL PAPER

Phosphorus enrichment enabled *Amorpha fruticosa* to invade on the floodplain of the Tagliamento River, Italy

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Abstract The North American nitrogen-fixing shrub *Amorpha fruticosa* (false indigo) is an aggressive invader of riverine habitats in Europe, though the reasons for its success are poorly understood. We hypothesized that its spread on the floodplain of the Tagliamento River in Northern Italy was enabled by anthropogenic phosphorus (P) enrichment. To investigate this hypothesis, we surveyed seed production at different locations along the river and performed a growth chamber experiment in which seedlings of three common floodplain shrubs (*A. fruticosa*, *Salix eleagnos* and *Buddleja davidii*) were grown at 10 levels of both nitrogen (N) and P. As a bioassay of N and P availabilities, we analyzed concentrations of these nutrients in *Salix eleagnos* leaves collected at different positions along the river. P availability was significantly higher in the lower reaches of the river, where *A. fruticosa* was abundant, than at its upstream limit. Numbers of *A. fruticosa* seeds per inflorescence increased strongly in a downstream direction and there was a trend for higher seed weight. In the growth experiment, *A. fruticosa* was more P-demanding than the other species, producing little biomass and no rhizobial nodules at low P. It also exhibited greater plasticity than the other species in both root mass fraction and ratio of longest root length to root mass. We conclude that anthropogenic P enrichment enabled *A. fruticosa* to invade what was originally a very oligotrophic environment. This N_2-fixing shrub exhibits greater phenotypic plasticity than native *S. eleagnos*, giving it a competitive advantage under conditions of high P availability.

Keywords Nitrogen-fixing shrub · Alien invasive plant · Seed production · *Amorpha fruticosa* · *Salix eleagnos* · *Buddleja davidii*

Introduction

Anthropogenic nutrient enrichment may enable fast-growing alien plant species to establish and spread (Daehler 2003; Lannes et al. 2016). This is most likely to occur in formerly nutrient-poor habitats, where no native species can benefit from, or even tolerate, high soil nutrient concentrations (Schumacher et al. 2009). In a study comparing the ecological traits of native and invasive alien plant species in Northern Italy, Dalle Fratte et al. (2019) found that the alien species occupied broadly similar ecological niche spaces to native species, but were more competitive

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(as assessed using CSR life-strategy theory; Grime 2006) and associated with more productive habitats. The authors concluded that, due to increased nutrient loadings (especially of N and P), plant communities in Southern Europe are increasingly dominated by species with more competitive growth strategies (Zhang et al. 2022).

Some of the most aggressive invaders of nutrient-poor ecosystems around the world are various shrubs and trees in the family Fabaceae (e.g. *Falcataria moluccana*, *Prosopis juliflora*, *Robinia pseudoacacia*, *Ulex europaeus, Spartium junceum*; Global Invasive Species Database 2022). One of these is *Amorpha fruticosa*, which was introduced from North America to Europe, where it now flourishes in a wide range of habitats including river floodplains and riparian forests (Kozuharova et al. 2017; Radovanović et al. 2017; Grabić et al. 2022).

One reason for the success of fabaceous species is their symbiosis with N_2-fixing bacteria, which supply a large proportion of their N requirements and enable rapid growth even under nitrogen-poor conditions. For example, N_2-fixation rates in the range 95–179 kg N ha^{-1} yr^{-1} have been reported in stands of *A. fruticosa* (USDA Plants Database https://plants.usda.gov/home) and < 112 kg N ha^{-1} yr^{-1} in *Robinia pseudoacacia* (Vítková et al. 2015). Furthermore, by increasing N availability, these species can facilitate the entry of other fast-growing plants, thus bringing about cascading changes in the vegetation (Kaur et al. 2012; Blaser et al. 2013; Cavieres 2021). In a study of riparian grasslands in Northern Italy, invasion by *A. fruticosa* was associated with increased rates of soil mineralization and nitrification, reduced light and lower floristic diversity (Boscutti et al. 2020).

Despite these examples, the capacity to fix atmospheric N_2 is not always advantageous for plants growing in nutrient-poor soils. This is because N_2 fixation is a P-demanding process (Binkley et al. 2000; Valentine et al. 2017; Vitousek et al. 2002) and may be restricted in sites where this nutrient is in short supply (Crews 1999; Eisele et al. 1989). The establishment phase is especially critical because future growth depends upon the seedling forming functioning nodules, which may only be possible if there are adequate reserves of P in the seed. In the common bean, *Phaseolus vulgaris*, for example, a high seed P concentration was shown to enhance nodulation and N_2 fixation in seedlings, making the young plants less dependent on soil P supply (Chagas et al. 2010). In the tropical woody legume *Leucaena leucocephala*, P reserves in the seed were found to support seedling growth for at least 31 days and were important in establishing the N_2-fixing symbiosis before mycorrhizal P uptake was sufficiently established (Slot et al. 2013). Producing seeds with adequate P reserves, however, incurs high P costs, which might reduce the reproductive output of plants growing in P-poor soils. These factors may explain why low soil P often restricts woody legumes from the poorest soils (Crews 1999; Vitousek et al. 2002).

Many river floodplains have been heavily invaded by woody plants, including by several N_2-fixing species (Höfle et al. 2014). Amongst the factors known to make these habitats vulnerable to invasion are frequent disturbance through flooding, transport of propagules by the river (e.g. Drescher and Prots 2016; Wagner et al. 2020), and reductions in flow due to abstraction and regulation (e.g. Cooper et al. 2003; Kuzmina et al. 2022). However, relatively few studies have examined the role of nutrient enrichment in driving plant invasions, despite the fact that river floodplains are often large sinks for anthropogenic nutrients, especially N and P (Gordon et al. 2020).

Here, we investigate the possible influence of P availability on the occurrence and performance of the invasive alien shrub *A. fruticosa* L. (Fabaceae) on the floodplain of the Tagliamento River in northern Italy. A survey of woody plants on the active floodplain of the River Tagliamento (Karrenberg et al. 2003) showed that *A. fruticosa* was restricted to the lower reaches of the river, where it was often very abundant and occupied similar sites to the native shrub *Salix eleagnos*. Other studies on the Tagliamento have shown that the water in the upper reaches is extremely poor in P, but that concentrations rise considerably in the lower reaches, probably because of contamination from agriculture and settlements (Arscott et al. 2000; Kaiser et al. 2004). Based on these findings, and the fact that many fabaceous species have a high P requirement, we hypothesized that a shortage of this nutrient has restricted *A. fruticosa* to the lower reaches of the Tagliamento River.

To investigate this hypothesis, we used a combination of field and experimental studies. The field studies were designed to determine how phosphorus availability affected reproductive performance of *A. fruticosa* plants along the Tagliamento. For this, we

measured various aspects of seed production at different locations along the river, including the quantities of P and N invested in seeds. Soil analysis could not be used to determine nutrient availability, because the floodplain was composed of coarse gravel and the shrubs had very deep root systems. We therefore used a bioassay approach, measuring N and P concentrations in the leaves of the common native shrub *Salix eleagnos*. The controlled environment experiment was designed to investigate the effects of N and P supply on the growth of *A. fruticosa* seedlings. For comparison, we included two other species in the experiment - *S. eleagnos*, as the most widely distributed native shrub species on the Tagliamento floodplain, and *Buddleja davidii*, as an abundant non-native species that lacks any N_2-fixing symbiosis. In the discussion we evaluate the hypothesis that P limits the abundance and range of *A. fruticosa* on the Tagliamento and consider the broader implications of our findings for invasion biology.

Methods

Study species

Amorpha fruticosa (false indigo bush) is a multi-stemmed shrub that grows < 5 m tall. It produces numerous dense spikes or racemes, each with 100–200 purplish blue flowers. The indehiscent fruits (pods) usually contain a single seed weighing 5–8 mg, though pods producing two seeds have been reported in the literature. In addition to producing abundant seed, *A. fruticosa* reproduces vegetatively by suckers and from stem fragments. Its native range in North America extends from central to eastern Canada south throughout much of the USA and into northern Mexico. It can tolerate soils ranging from relatively dry to moist, and grows in moist open woodland areas, floodplains, stream banks and swamp margins (CABI Compendium 2016).

Amorpha fruticosa was introduced into Europe as an ornamental and honey plant, and also to stabilize steep slopes such as railway embankments. It now occurs in a wide range of habitats, including riparian and alluvial habitats, sandy banks of ravines, coastal areas, dunes and disturbed land (Kozuharova et al. 2017). It is especially vigorous in periodically flooded sites such as the riparian forests of major rivers including the Danube, Sava, Tisza and Tagliamento, where it may grow so densely as to exclude all other plants (Radovanović et al. 2017; Grabić et al. 2022). Both in its native and introduced ranges, *A. fruticosa* is commonly infested by the seed beetle *Acanthoscelides pallidipennis*.

Study area

The Tagliamento River in northeastern Italy flows from the Julian and Carnian Alps across the Friulian Plain to enter the Mediterranean Sea east of Venice. With a total length of around 150 km, it has been described as the last morphologically intact river in the Alps (Fig. 1; Tockner et al. 2003; Ward et al. 1999). Because of steep slopes and huge reserves of sediment in the upper reaches, intense rain can generate high floods and massive sediment transport. As a consequence, for most of its length the Tagliamento has a wide and extremely dynamic floodplain that supports a changing mosaic of aquatic and terrestrial habitats. Downstream of Pinzano (about 94 km from the source), most of the surface flow is lost through infiltration into a vast alluvial aquifer dominated by highly permeable gravel. Some of this water returns to the river downstream of about km 120. Levels of dissolved P are very low along the entire length of the river (Arscott et al. 2000; Tockner et al. 2003; Kaiser et al. 2004), but particulate P increases markedly from 2 to 3 µg l^{-1} in the upper reaches to 12–26 µg l^{-1} below about km 74 (Arscott et al. 2000).

The active floodplain is mainly composed of bare gravel with scattered shrubs, though some reaches have numerous vegetated islands and riparian forest occur along the margins in places. Karrenberg et al. (2003) surveyed the woody vegetation of the floodplain in 13 segments of 1 km length located at 10 km distances along the river. The most frequent species were *Populus nigra, Alnus incana* and *Salix eleagnos*, which occurred in all or almost all segments. Other common species were *Frangula alnus, Fraxinus ornus, Hippophaë rhamnoides, Ligustrum vulgare* and various *Salix* species (*S. alba*, *S. daphnoides*, *S. purpurea, S. triandra*). *A. fruticosa* was common in the lower reaches of the Tagliamento and partial canonical correspondence analysis showed it to have the most downstream center of distribution of any woody species. Based on our own observations in 2022, the distribution of *A. fruticosa* along

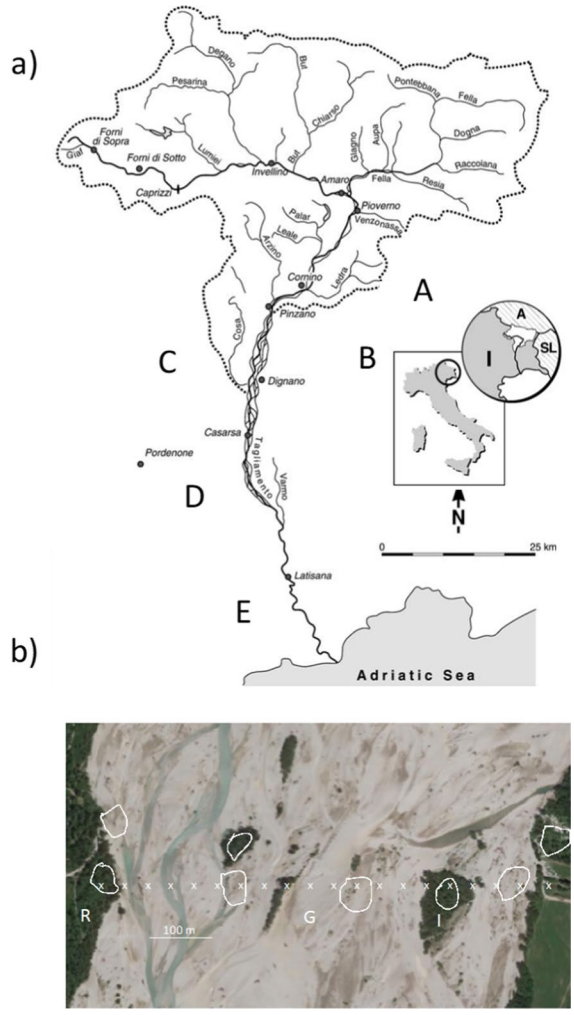


Fig. 1 **a** Catchment map of the Tagliamento River, with major tributaries and towns. Inset shows the location of the river in Italy (I), near the borders of Austria (A) and Slovenia (SL). The letters A–E show the sampling sites described in this study. Modified after Ward et al. 1999; **b** Sampling scheme, showing the transect of 20 points for sampling *Salix eleagnos* foliage, and 8 patches (4 in woodland, 4 on gravel) for sampling *A. fruticosa* inflorescences. *R* Riparian woodland, *I* Wooded island, *G* Gravel floodplain

the Tagliamento has scarcely changed in the 20 years since the survey of Karrenberg et al. (2003).

We studied the growth of *A. fruticosa* at five sites (A–E) on the active floodplain located between 76 and 136 km from the source of the Tagliamento (Fig. 1, SI Table 1). Site A was chosen as the uppermost site where *A. fruticosa* was regularly present on the floodplain. Above that point, a few plants could be found, mainly in special situations such where garden rubbish was dumped or where a channel joins the river. However, the species was fairly common on roadsides and wasteland as far upstream as the town of Tolmezzo, suggesting that its absence from the floodplain was not due to a lack of seed. It also indicated that the species was not excluded from upstream sites by adverse climatic conditions. The lowermost site E was chosen to represent the river downstream of the braided channel, where the river was confined within embankments. *A. fruticosa* was very abundant here, forming a dense understorey beneath taller trees.

Previous studies have described many types of vegetation on the floodplain, reflecting the wide diversity of substrates, topographic situations and successional stages (Lippert et al. 1995; Müller 2005; Kollmann et al. 1999; Karrenberg et al. 2003). For convenience, we made a simple distinction between plants growing in mainly bare gravel habitats and those growing in woody vegetation, including woodland with *Populus nigra*, riparian forest dominated by *Alnus incana* and *Fraxinus excelsior*, and scrub woodland dominated by various species. In general, woody vegetation was associated with accumulations of fine sediment and organic matter, as occurred in wooded islands and riparian woodland (Edwards et al. 1999; Gurnell et al. 2001), while the gravel habitats had very little fine sediment or organic material. In a survey of alien plant species on the floodplain of the Tagliamento, Altenburger and Ott (unpublished), found that *A. fruticosa* formed extensive stands in riparian forests and on islands but occurred only in small clumps and as scattered individuals on the active floodplain. Since there was no evidence of regeneration from seed on the floodplain, the authors concluded that these plants probably established from stem fragments originating in the forest and island populations.

Foliar nutrient concentration of Salix eleagnos

Many studies have found strong positive relations between nutrient concentrations in leaves, especially of N and P, and the availability of these nutrients in the soil (Atkinson 1973; Hofmeister et al. 2012; Millard and Proe 1993; Vitousek 1998). In an experiment conducted in stands of six northern hardwood species in New Hampshire USA, Hong et al. (2022) found that adding nutrients increased foliar concentrations by an average of 12% for N

Table 1 Concentrations of nitrogen and phosphorus in the culture solutions used in the growth experiment and the amounts applied to plants over 8 weeks (5 ml culture solution per week)

Nitrogen series (0.533 mg P / plant)			Phosphorus series (8 mg N / plant)		
Level	mg N / 500 ml	N / plant (mg)	Level	mg P / 500 ml	N / plant (mg)
1	1.6	0.125	1	0.1	0.008
2	3.1	0.25	2	0.2	0.017
3	6.3	0.5	3	0.4	0.033
4	12.5	1	4	0.8	0.067
5	25	2	5	1.7	0.133
6	50	4	6	3.3	0.267
7	100	8	7	6.7	0.533
8	200	16	8	13.3	1.066
9	400	32	9	26.7	2.132
10	800	64	10	53.3	4.262

and 45% for P, with some variation in the response among species. Studies of this kind have encouraged the widespread use of foliar concentration data as a convenient proxy for soil nutrient status (Hong et al. 2022; Townsend et al. 2007). In our study, conventional soil analysis was not possible for two reasons: first, the Tagliamento floodplain was composed mainly of coarse gravel, with varying amounts of interstitial sand where the roots could penetrate; second, most woody plants had very deep root systems, making it difficult to collect samples from an ecologically relevant depth. For these reasons, we measured P and N concentrations in leaves of *Salix eleagnos* as an indicator of nutrient availability for woody plants. This species was chosen because it was by far the commonest shrub on the floodplain and often grew in close proximity to *A. fruticosa.*

S. eleagnos leaves were sampled at each site in June 2010, by locating 20 equally spaced points along a transect across the floodplain. The exact spacing between the sampling points depended on the width of the floodplain, but was usually between 30 and 40 m. Within 10 m of each point, we selected a *S. eleagnos* shrub that was > 1 m high and had a stem diameter > 1.5 cm, if such a plant was present. If insufficient samples were collected from the transect, we set out a second parallel transect about 50 m away. Site E was not sampled, because *S. eleagnos* was very scarce in the channelized section of the river. A total of 96 samples were obtained for chemical analysis (average of 12 samples per site and habitat). The samples were dried, milled and, following Kjeldahl digestion, analyzed for total N and P contents using a continuous flow injection analyzer (AutoAnalyzer 3 h; Seal Analytical, Southampton, UK).

Seed production by *A. fruticosa*

The fieldwork for this part of the study was conducted in mid-March 2022, when the inflorescences still bore the dry pods from the previous year. *A. fruticosa* was distributed less uniformly across the floodplain than *S. eleagnos*, and a different sampling scheme was therefore needed. At each site, for gravel and woodland habitats separately, we located patches where several plants could be found growing < 50 m apart, with adjacent patches being separated by at least 100 m. In each patch, we collected the five longest inflorescences (taking only one inflorescence per plant), which together constituted a sample. Our goal was to collect 8 samples per site, four from woodland and four from gravel; this was not possible at Site A, where there were too few plants on the gravel, and at site E, where the gravel floodplain was altogether absent. In total, 34 samples were obtained.

In the laboratory, the pods were removed from the inflorescences and weighed. For each sample, 100 pods were dissected to remove the seeds and the healthy seeds were counted and weighed. In this way, we obtained the following parameters: mean number of pods per inflorescence, proportion of seeds infested by the seed beetle *Acanthoscelides pallidipennis* or otherwise damaged, and mean seed weight. The healthy seeds were analyzed for P and N. To investigate the influence of seed P and N reserves upon early seedling growth, we germinated healthy seeds from 20 samples and grew them in quartz sand watered

with distilled water. After 35 days the seedlings were harvested, dried at 70 °C and weighed.

Growth responses of three floodplain shrubs to P and N

In a controlled environment experiment, we investigated the effect of N and P supply on seedling growth of *A. fruticosa*. For comparison, we included two other species—*S. eleagnos*, the most widely distributed native shrub species on the Tagliamento floodplain, and *B. davidii*, an abundant non-native species with no N_2-fixing symbiosis. Seeds of the three species were collected from the Tagliamento floodplain and sown in trays. The young seedlings were then transplanted to 1.1 l containers filled with 0.7–1.2 mm quartz sand.

From stock solutions, we prepared two series of 10 solutions containing all essential nutrients but with different levels of either N or P (Table 1). Plants in the N series received one of 10 N levels and a fixed level of P (0.533 mg P per plant), while plants in the P series received one of 10 P levels and a fixed level of N (8 mg N per plant). These nutrients were applied in 8 weekly doses (5 ml of the various nutrient solutions), with water being added as needed. The experiment was conducted in a climate chamber with day/night temperature regime of 24 °C/20°C and a day length of 16 h. The light intensity was 450 µmol m^{-2} s^{-1}.

Plants were harvested after 8 weeks. The longest root of each plant was measured and, for *A. fruticosa*, all root nodules were counted. Roots and shoots and nodules were then separated, dried to constant weight at 70 °C and weighed. From these measurements, we obtained total dry weight, root mass fraction (RMF) and length of the longest root divided by root dry weight (RLM; this parameter was used as a substitute for specific root length—i.e. total root length to root mass, which proved to be too time-consuming to measure).

Statistical analysis

The data for foliar N and P contents in *S. eleagnos* and seed production by *A. fruticosa* were analyzed using two-way analysis of variance, with distance along the river and habitat type (gravel and woodland) as the grouping factors. For parameters showing a significant distance effect, pairwise comparisons among sites were tested using Tukey's HSD test. Pearson's correlation coefficients were calculated for all pairs of seed parameters.

In the growth experiment, the strength of growth responses to varying P and N concentrations was quantified by calculating coefficients of variation (CV = 100*SD/mean) for each parameter across the 10 nutrient levels in each series. We also calculated the goodness of fit (R^2) and significance levels for third order polynomial curves fitted to the data for each parameter.

All analyses were performed using R version 4.2.1.

Results

Nitrogen and phosphorus content of *Salix eleagnos* leaves

A two-way analysis of variance showed that P concentrations in the leaves of *S. eleagnos* varied significantly among the four sites ($P < 0.001$; Fig. 2, Supplementary Materials for details of ANOVA's), but not between the gravel and woodland habitats ($P > 0.05$). On gravel, mean foliar P increased from 0.91 mg P g^{-1} at site A (76 km from source) to 1.41 mg P g^{-1} at site D (111 km). Foliar P also increased from sites A to C in the woodland samples, while the mean value at site D was rather low (1.07 mg P g^{-1}). Foliar N concentrations also varied significantly among the four sites ($P < 0.01$), but without a clear downstream trend, and were significantly higher in woodland than on gravel (12.2 v. 10.1 mg N g^{-1}, respectively; $P < 0.001$). N:P ratios in leaves varied significantly, both among the four sites and between the two habitat types (both $P < 0.001$); the mean N:P ratio on the floodplain fell from 11.8 at site A to 6.7 at site D, while the mean values on gravel and in woodland were 9.2 and 11.2, respectively.

Seed production in *A. fruticosa*

The mean number of pods per inflorescence increased significantly (2-way ANOVA: $P < 0.001$) in a downstream direction, from 175 at site A to 292 at site E (Fig. 3a). Number of pods was also significantly higher in woodland than on gravel ($P < 0.01$). Many pods, all containing a single seed, were infested by

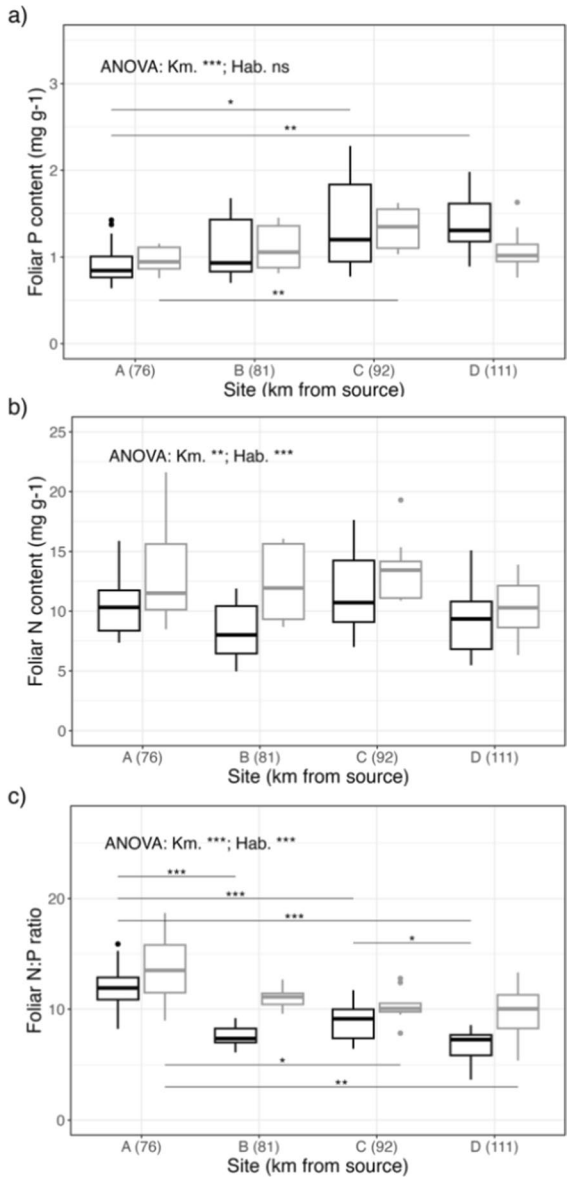


Fig. 2 Nutrient analyses of *Salix eleagnos* leaves from the floodplain of the Tagliamento River, Italy: **a** N concentrations, **b** P concentrations, **c** N:P ratios. Black bars-gravel; grey bars-woodland. Significance of 2-way ANOVA's for river kilometre and habitat are also shown: +, *, **, *** – $P<0.1$, 0.05, 0.01, 0.001, respectively; *ns* Not significant; significant pairwise differences between sites are shown by horizontal lines. Details of ANOVA's in Supplementary Materials Table S2

the beetle *Acanthoscelides pallidipennis*, while some seeds were blackened or misshapen and almost certainly not viable. There was a trend for the mean percentage of apparently healthy seeds to decrease downstream ($P=0.08$), from 87% at site A to 75% at both sites D and E. The percentage of healthy seeds also varied according to habitat, being significantly lower in woodland than on gravel ($P<0.05$).

Mean seed weight was lowest at site A (5.3 mg) and highest at site E (6.7 mg), but the downstream trend did not reach the level of statistical significance ($P=0.08$). Seed P and N concentrations were positively correlated (r = 0.59, $P<0.001$, $n=34$) and varied considerably among samples, but this variation was not related to either position along the river or habitat type (ANOVA; $P>0.05$). Mean concentrations (± SD) were 2.62 ± 0.38 mg P g^{-1} and 47.8 ± 3.4 mg N g^{-1}. There was also no significant variation in the P and N contents per seed with either position or habitat.

Seeds from 20 samples were germinated in quartz sand and raised without added nutrients. The seeds used in this experiment had a mean dry weight of 6.11 ± 0.14 mg and contained an average of 16.0 ± 3.6 µg P and 295 ± 54.0 µg N. After 35 days, the seedlings had a mean dry weight of 17.8 ± 6.13 mg, which was 2.92 times greater than that of the seeds. Seedling dry weight was significantly correlated with both seed P (r = 0.65, $P<0.005$, $n=20$) and N contents (r = 0.55, $P<0.02$), but not with seed dry weight (0.43, $P>0.08$). There was also a significant positive correlation between seedling dry weight and position along the river (r = 0.62, $P<0.01$).

Growth responses of three floodplain shrubs to P and N

In the growth experiment, *S. eleagnos* produced the largest plants overall (mean total biomass ± SD over all treatments was 0.8 ± 0.33 g; Fig. 4), followed by *B. davidii* (0.37 ± 0.20 g) and *A. fruticosa* (0.22 ± 0.23 g). The species also differed greatly in biomass allocation, with *S. eleagnos* producing the highest root mass fraction (mean RMF 0.60 ± 0.072), followed by *B. davidii* (0.45 ± 0.069) and finally *A. fruticosa* (0.34 ± 0.091). Ratio of longest root length to root mass (RLM) was highest for *A. fruticosa* (277 ± 141 m g^{-1} dry weight), followed by *B. davidii* (101 ± 54 m g^{-1}) and then *S. eleagnos* (55 ± 17 m g^{-1}).

Amorpha fruticosa responded strongly to P (CV 134%; Table 2), producing most biomass at level 10 and very little biomass at P levels of 5 and less. In

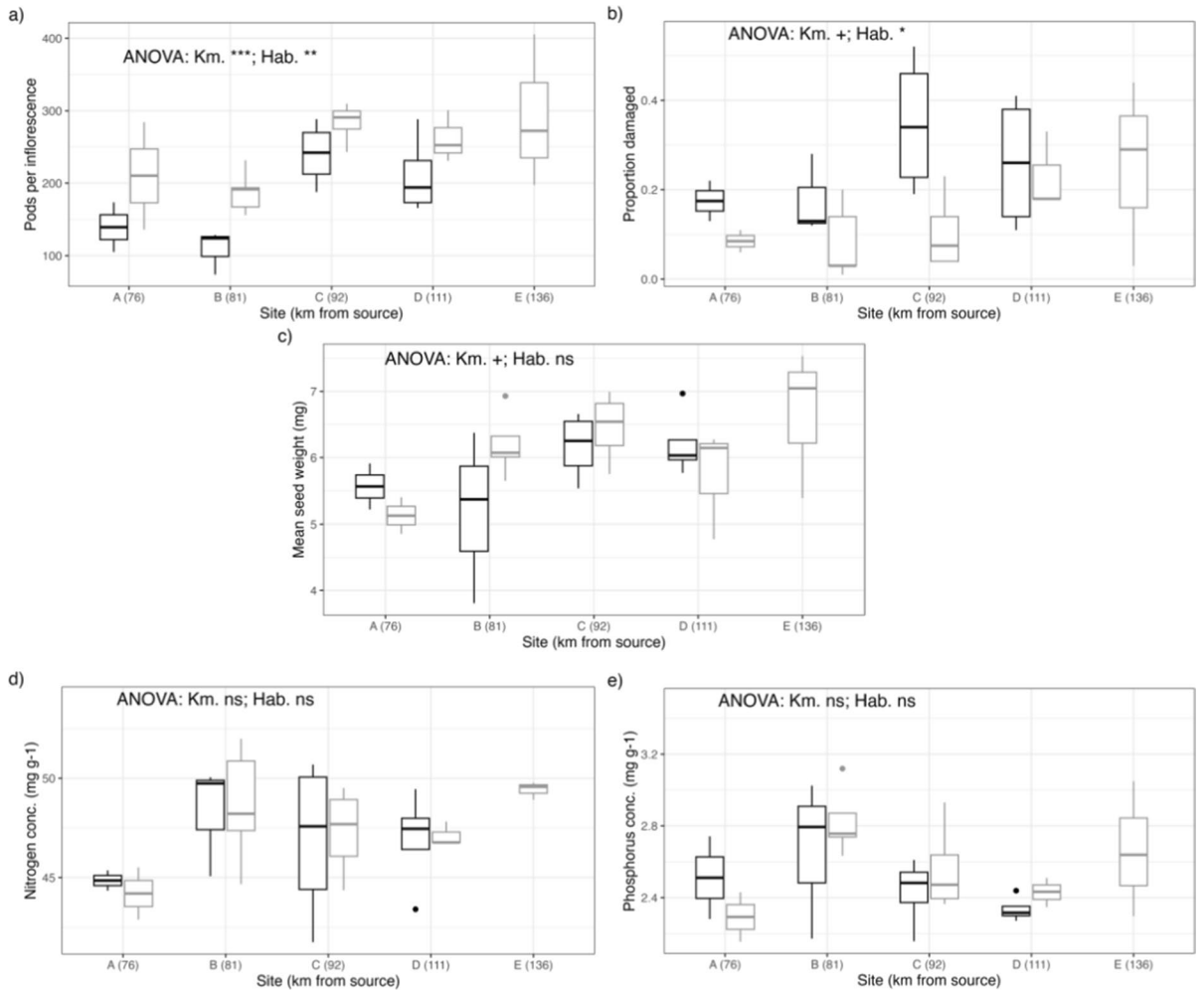


Fig. 3 Seed production by *A. fruticosa* on the floodplain of the Tagliamento River, Italy: **a** Number of pods per inflorescence, **b** Proportion of damaged seeds, **c** Mean seed weight, **d** Seed N concentration, **e** Seed P concentration. Black bars-gravel; grey bars-woodland. Significance of 2-way ANOVA's for river kilometre (Km.) and habitat (Hab.) are also shown: +, *, **, *** – $P < 0.1$, 0.05, 0.01, 0.001, respectively; *ns* Not significant. Details of ANOVA's are given in Supplementary Materials Table S3

the highest P treatment, it produced more shoot biomass than *S. eleagnos*, despite having a lower total biomass. Growth of *A. fruticosa* varied less in the N series (CV 41.4%), being rather consistent over levels 1 to 8 but markedly lower at the highest two levels (Table 2).

S. eleagnos also showed a clear response to P (CV 49.1%; Table 2), with peak biomass at level 9 and some growth even at the lowest P level. In the N series, highest biomass production was at high N (level 8), but with some growth even at level 1 (CV 33.1%). Results for *B. davidii* were less clear, partly because of poor growth of individual plants. In the P series, growth was highest at level 10 and very poor at level 1 (CV 69.1%; Table 2). In the N series, growth varied rather little across levels but was poorest at level 10 (CV 45.3%).

In *A. fruticosa*, RMF varied considerably across P levels (CV 33%), with a clear peak at level 4. In contrast, N level had relatively little effect on RMF (CV 19.4%), except for a low value at level 1 and a high value at level 10. RMF varied rather little in *S. eleagnos* and *B. davidii*, with CV values of 10.6 and

16.1%, respectively, in the P series, and 11.3% and 13.9% in the N series.

Root length to mass ratio (RLM) was generally higher at low P (levels 1–4) than at high P (levels 7–10) in all species. Variation in RLM was much greater in *(A) fruticosa* (CV 54.1%) than in *B. davidii* and *S. eleagnos* (36.8 and 33.5%, respectively). There were no consistent trends in RLM in the N series.

Numbers and mass of root nodules in *A. fruticosa* varied greatly in both nutrient series. In the P series, most nodules were produced with high P, and very few nodules were produced in levels 1–6 (Fig. 5). In the N series, very few nodules were produced at high N (levels 8–10), and the highest nodule mass was in the treatment with the lowest N.

Discussion

Nutrient availability on the floodplain of the Tagliamento River

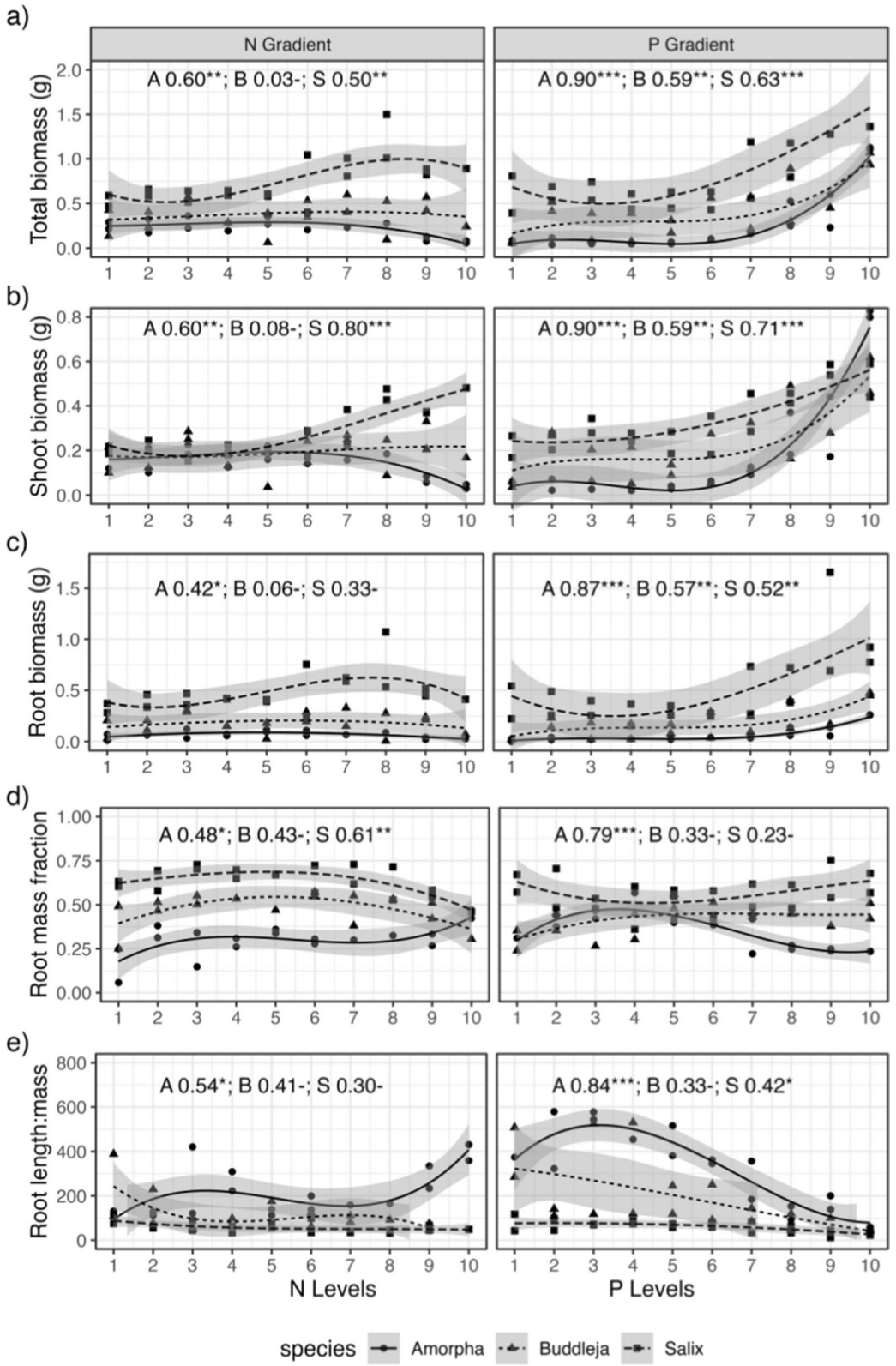
We investigated the hypothesis that invasion of the lower reaches of the Tagliamento River by the alien N_2-fixing shrub *A. fruticosa* was enabled by anthropogenic P enrichment. Our first task was to establish whether P and N availabilities to woody plants varied along the floodplain, which we inferred from concentrations of these nutrients in *Salix eleagnos* foliage. Various points emerge from these data. First, measured N and P concentrations were at the lower end of the ranges reported for deciduous shrubs and trees (Aerts and Chapin 1999) and far lower than those for 13 floodplain species of *Salix* (mean values ± SD for data from the TRY database: 25.0 ± SD 3.5 mg N g^{-1}; 2.27 ± 0.59 mg P g^{-1}). Second, foliar P in the gravel habitat increased strongly in a downstream direction, being 55% higher at site D than at site A (1.41 v 0.91 mg P g^{-1}). In contrast, N concentrations showed no clear downstream trend and the recorded decline in the N:P ratio-from 12.4 at site A to 8.5 at site D – was largely due to the increase in P. Third, it was clear that other factors also influenced foliar nutrient concentrations. One of these was habitat type, with mean N concentrations being consistently higher in woodland than on the open gravel floodplain. Another was probably drought, which most affected the vegetation in reaches where the water flows deep beneath the gravel, including site D. Drought is known to reduce the bioavailability of soil phosphorus (Zhang et al. 2020), which could explain the lower than expected P concentrations in leaves from woodland at site D.

Information about the P status of the Tagliamento and its floodplain can also be gleaned from several previous studies. These indicate that the entire river system was originally very nutrient poor, which is reflected in exceptionally low P concentrations in the water, especially in the upper reaches (Kaiser 2002), an aquatic biota that is adapted to highly oligotrophic conditions (Arscott et al. 2000), and nutrient-poor vegetation in the upper reaches (Karrenberg et al. 2003). Today, however, large quantities of P enter the system from the many towns within the catchment and from intensively used farmland, especially downstream of Pinzano (km 94).

Seed production in *A. fruticosa*

With a mean of 2.62 ± 0.38 mg g^{-1}, the P concentration in *A. fruticosa* seeds was at the lower end of the range reported for leguminous species (e.g., concentrations between 3.1 and 7.5 mg g^{-1} reported in Bolland and Paynter 1990, Embaby and Rayan 2016, and Wang et al. 2020). In contrast, the mean seed N concentration was 47.8 ± 3.4 mg g^{-1}, which is typical of the relatively high values found in other N_2-fixing leguminous species (e.g. Wang et al. 2020). As a consequence, the mean N:P ratio (18.5 ± 1.91) was considerably higher than the mean value of 11.3 reported for 57 N_2-fixing species in an alpine meadow in Tibet (Wang et al. 2020). From these comparisons, we might expect the early growth of seedlings to be limited more by seed reserves of P than of N. This idea is supported by the results of the germination experiment in which seedlings were raised for 35 days without added nutrients: in that experiment, growth correlated more strongly with the P than with the N content of seeds, and was uncorrelated with seed dry weight.

Seed weight increased in a downstream direction, though only a small proportion of the variation was explained by this factor. However, this result was consistent with an earlier unpublished survey in which pod weight was found to correlate significantly with distance downstream ($R = 0.227$, $P < 0.01$, $n = 129$). In contrast, seed P and N concentrations showed no significant relationship to either position along the river or habitat, despite the evidence from foliar


a)
N Gradient
P Gradient
Total biomass (g)
A 0.60**; B 0.03-; S 0.50**
A 0.90***; B 0.59**; S 0.63***
b)
Shoot biomass (g)
A 0.60**; B 0.08-; S 0.80***
A 0.90***; B 0.59**; S 0.71***
c)
Root biomass (g)
A 0.42*; B 0.06-; S 0.33-
A 0.87***; B 0.57**; S 0.52**
d)
Root mass fraction
A 0.48*; B 0.43-; S 0.61**
A 0.79***; B 0.33-; S 0.23-
e)
Root length:mass
A 0.54*; B 0.41-; S 0.30-
A 0.84***; B 0.33-; S 0.42*
N Levels
P Levels
species
Amorpha
Buddleja
Salix

◂**Fig. 4** Growth of seedlings of three floodplain shrubs in culture solutions with 10 levels each of N and P: **a** total biomass, **b** shoot biomass, **c** root biomass, **d** root mass fraction (RMF), **e** ratio of length of longest root to root biomass (RLM). Points show individual plants and curves show fitted third order polynomials with 95% confidence intervals. *Amorpha fruticosa* (A) circles, solid lines; *Buddleja davidii* (B) triangles, fine dashed lines; *Salix eleagnos* (S) squares, coarse dashed lines. Goodness of fit (R^2) and significance levels of the polynomial curves are also shown: - not significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. See Methods and Table 1 for quantities of N and P applied at each level

analysis of increasing P availability downstream and higher N availability in woodland than on the gravel floodplain. Overall, our results suggest that the dry weight and nutrient contents of *A. fruticosa* seeds are relatively constant, with seeds of similar quality being produced under widely different conditions. In contrast, number of seeds produced per inflorescence was highly variable, with many more seeds being produced downstream than upstream, and more in woodland than on gravel. Although seed predation by *Acanthoscelides pallidipennis* increased in a downstream direction, this effect was not sufficient to cancel out the generally higher seed yields downstream.

A limitation of our study is a lack of direct evidence that P availability was the main factor limiting seed production. Indeed, it is likely that other factors also influenced the number, size and nutrient content of seeds. Climatic conditions can probably be ruled out, because *A. fruticosa* is common in the region, growing in disturbed sites and roadsides well beyond its distribution limit on the floodplain. However, very dry soils may explain low seed weights at site D.

Despite this limitation, two observations are worth mentioning, which - while not conclusive - are consistent with the P hypothesis. The first is that several plants at sites A and B bore rather lax inflorescences that looked different from the usually dense spikes of *A. fruticosa*. Closer examination revealed that many flowers in these inflorescences had not developed, leaving only their pedicels between widely spaced pods. These unusual inflorescences may be the result of *A. fruticosa* adjusting its seed output to prevailing nutrient conditions. Such regulation of seed number through abortion of flowers, pistils or ovules has been reported for many other species (Brevedan et al. 1978; Haberman et al. 2021).

The second observation is that, in contrast to low seed production towards the upstream limit of *A. fruticosa*, reproductive output at downstream sites was sometimes very large. For example, a 1×1 m^2 area sampled at site E yielded 238 g of pods that contained an estimated 0.43 g P and 8.03 g N. On a per hectare basis (4.3 kg P ha^{-1}, 80.3 kg N ha^{-1}), these quantities of nutrients are within the ranges harvested in agricultural crops, which can only be sustained using fertilizer. Along the Tagliamento, such a high P allocation to reproduction would probably only be possible in areas of considerable enrichment.

Growth responses of three floodplain shrubs to P and N

The growth experiment revealed strongly contrasting responses of the three species to N and P supply. In the P series, *A. fruticosa* was the most demanding species, producing a high biomass with the highest P concentration and almost no biomass with the five lowest concentrations. This failure to thrive at low P can be linked to the absence of root nodules, which would also have reduced the seedlings' N supply. *A. fruticosa* was very plastic in both root mass fraction (RMF) and ratio of longest root length to root mass (RLM), both of which were higher at medium or low P than at high P. In the N series, *A. fruticosa* grew better with a low than with a high N supply.

S. eleagnos showed a more muted response to P, producing most biomass at higher P concentrations (but not the highest), and maintaining a moderate level of growth even at the lowest P concentration. It was also much less plastic in response to P, with RMF remaining relatively constant across the range of P concentrations and RLM increasing somewhat at low P. In the N series, *S. eleagnos* grew best at higher N concentrations (levels 2–5), but maintained some growth even at the lowest level.

The results for the non-native shrub *B. davidii* were in many respects intermediate between those for *A. fruticosa* and *S. eleagnos*. As for *A. fruticosa*, growth was strongest at highest P and very poor at the lowest P concentration. However, plasticity in RMF and RLM, as reflected in CV's, was only slightly greater than in *S. eleagnos*.

From these results, two main conclusions can be drawn concerning the invasive legume *(A) fruticos*a. Firstly, this species has a much higher P requirement than either the native *S. eleagnos*, which occupies a similar niche on the floodplain of the Tagliamento

Table 2 Coefficients of variation (CV %) for parameters measured in the growth experiment. Each CV uses the mean values for each of 10 nutrient concentrations in the P or N series

Parameter	*Amorpha fruticosa*		*Buddleja davidii*		*Salix eleagnos*	
	P series	N series	P series	N series	P series	N series
Total biomass	134	41.4	69.1	45.3	49.1	33.1
Root mass fraction (RMF)	33	19.4	16.9	13.9	10.6	11.3
Longest root length/root mass (RLM)	54.1	36.7	36.8	42.1	33.5	28.9
Number of nodules	117	65.9				
Mass of nodules	196.5	136.4				

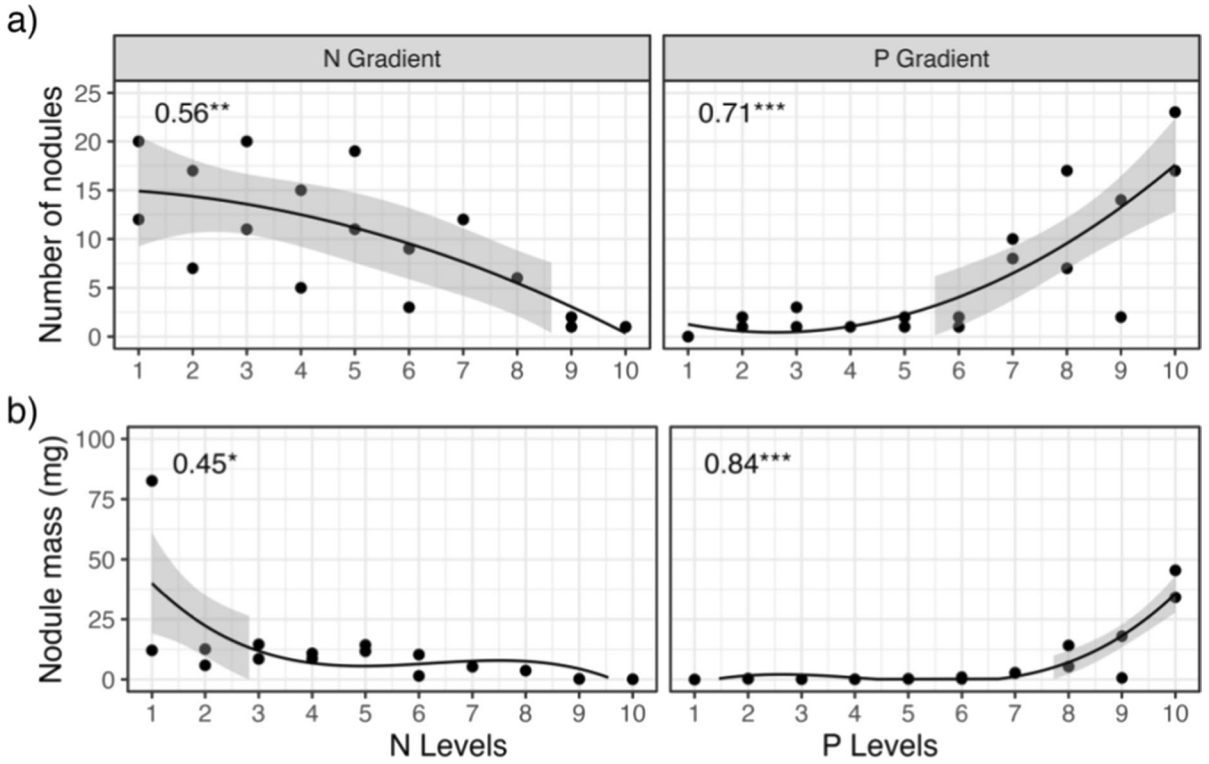


Fig. 5 Nodules of *Amorpha fruticosa* seedlings grown in culture solutions with 10 levels each of N and P: **a** number of nodules, **b** dry mass of nodules. Points show individual plants; curves show fitted third order polynomials with 95% confidence intervals. Goodness of fit (R^2) and significance levels of the polynomial curves are also shown: - not significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. See Methods and Table 1 for quantities of N and P applied at each level

River, or the alien invasive *B. davidii*. Other studies have also shown leguminous shrubs to have a relatively high P requirement (Crews 1999; Vitousek et al. 2002). Secondly, *A. fruticosa* shows greater phenotypic plasticity than either *S. eleagnos* or *B. davidii* in allocation of biomass to roots and in the ratio of root length to root mass. This finding is consistent with other studies showing that legumes generally exhibit high flexibility in traits related to N acquisition and assimilation, especially under conditions of P deficiency (Pons et al. 2007; Wolf et al. 2017; Valentine et al. 2017). In addition, high plasticity in traits related to resource capture appears to be common in invasive species (Daehler 2003; Richardson and Pyšek 2006; Schumacher et al. 2009); indeed, it is this characteristic of invasive species that gives them a competitive advantage over native species when resource availability increases, as in the case of

P along the Tagliamento. It is interesting to note that *A. fruticosa* only produced more shoot biomass than *S. eleagnos* at the highest P level, suggesting that this species may compete with native species by producing more abundant foliage, which can only happen under high P conditions.

Concluding remarks

The Tagliamento was originally a highly oligotrophic river that in historical times became increasingly enriched by P. The evidence presented here suggests that higher levels of P have created a new niche on the floodplain that has been occupied by the alien invasive legume *A. fruticosa*. Seed production of this species decreases strongly in an upstream direction and we speculate that its upper limit is set by insufficient seed production under phosphorus-poor conditions. In contrast, the alien species *B. davidii* is not restricted to the lower reaches of the Tagliamento, but is abundant along the entire length of the river. Our growth experiment shows that *A. fruticosa* has a higher P requirement and is more plastic in its growth responses to nutrients than both *B. davidii* and *S. eleagnos*. However, further field studies would be needed to confirm that P is the principal factor limiting the distribution of *A. fruticosa*.

A. fruticosa is not the only N_2-fixing shrub to invade river floodplains. *Robinia pseudoacacia*, a native of North America, is widely invasive in Europe, including on floodplains. Similarly, *Elaeagnus angustifolia*, which is native to western and central Asia, is highly invasive on floodplains in North America, often suppressing native *Salix* and *Populus* species (Lesica and Miles 2001). It is now becoming invasive in European wet habitats, especially in Austria (Lapin et al. 2019). Whether P enrichment plays a role in these invasions is unknown, but would be worth investigating. It is known, however, that both species—like *A. fruticosa* (Boscutti et al. 2020)—fix large amounts of atmospheric N_2, which can produce profound changes in both soil conditions and species composition of vegetation (see Buzhdygan et al. (2016) for *R. pseudoacacia* and DeCant (2008) for *E. angustifolia*). For this reason, floodplains that remain uninvaded by N_2-fixing shrubs—like the upper reaches of the Tagliamento - are especially worthy of protection, including measures to prevent eutrophication.

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest Costs of field and laboratory work were supported by the Geobotanical Institute ETH (Stiftung Rübel). The authors have no relevant financial or non-financial interests to disclose.

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