RESEARCH ARTICLE

Tradeoffs between productivity and nitrogen conservation in wild and domesticated plants of the perennial crop *Physaria* (*Brassicaceae*)

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Abstract

Domestication of desert-adapted perennials has been proposed as an alternative to increase cropping intensity in low input environments. However, selection for high yield in perennial crops provokes indirect changes in plant resource use. These changes might alter how nitrogen is acquired, allocated, used and stored, thus affecting plant longevity and yield stability. Using the perennial forb *Physaria* as a model, we compared the pattern of N allocation, use and conservation of high-yield accessions (Y), and stable yield accessions (S), with their wild counterpart (W). We found a negative relationship between seed yield and N conservation capacity and their associated traits. The shift of strategy provoked by high yield selection implies changes in N allocation and a decrease in nitrogen use efficiency and conservation capacity at plant- and leaf-level. S accessions also increase allocation to seeds, although they kept the N use and conservation traits of their wild counterparts, remaining in an intermediate position between the Y and W accessions profiles. The shift of strategy provoked by high yield selection implies a decrease in nitrogen use efficiency and conservation capacity that may be behind the loss of longevity and yield on subsequent years. Wild and stable accessions were more conservative and show traits that promote nitrogen conservation. These are key traits that should not be lost during the selection process if breeders want to achieve the adequate ideotype of perennial crop for arid systems.

Introduction

Domestication of perennial wild species and their development into crops has been proposed as an alternative to increase cropping ecological intensity in low-resource environments (Van Tassel *et al.,* 2017). This option might reduce nitrogen requirements and losses (Crews & Peoples, 2005; Asbjornsen *et al.,* 2014; Crews *et al.,* 2016) and increase yield stability (González-Paleo & Ravetta, 2011a) because perennials exhibit a suite of traits such as low relative growth rate, leaves with low nitrogen content, high leaf life span, small specific leaf area (SLA) and effective nutrient resorption that contribute to a low dependence on outer sources and decreases losses of nitrogen (Aerts, 1996; Aerts & Chapin, 2000; Wright *et al.,* 2002).

However, improvements in seed yield are frequently achieved sacrificing adaptive attributes of high ecological value (Van Tassel *et al.,* 2010; Denison, 2012; Vilela & González-Paleo, 2015). A general shift of strategy from conservative to acquisitive has been described as an indirect effect of high-yield selection (González-Paleo & Ravetta, 2011a; Milla *et al.,* 2014; Vilela & González-Paleo, 2015), including increases in SLA and carbon assimilation rate (Pujol *et al.,* 2008; González-Paleo & Ravetta, 2011a) and decreases in carbohydrate reserves and perpetuation structures (roots, rhizomes, rosette, etc.; Piper & Kulakow, 1994; Vico *et al.,* 2016). These changes might limit the capacity of perennials for water and nitrogen acquisition and conservation in subsequent years (Cox *et al.,* 1985; Foulkes *et al.,* 2009), reducing plant

longevity and compromising seed yield in low resource environments (González-Paleo & Ravetta, 2012).

We have developed domestication models based on C economy of perennial species in the genus *Physaria* comparing high seed-yield selected accession with their wild counterparts (González-Paleo & Ravetta, 2011a,b, 2012, 2015; Vilela & González-Paleo, 2015). However, lower attention has been paid to traits related to nitrogen use efficiency (NUE) and conservation, despite this nutrient is often the most limiting for growth in arid lands (Schlesinger *et al.,* 1996). The pattern of nitrogen allocation and the set of traits implied in its acquisition, conservation and use determine the capacity of a plant to survive and reproduce (Eckstein *et al.,* 1999). Thus, the understanding of changes in N economy because of selection for high yield or yield stability is central to define a perennial crop ideotype and the choice of selection criteria aimed to increase yield without a massive intensification in resource inputs (González-Paleo & Ravetta, 2012).

Storage and redistribution of N are important factors determining seed yield, next season regrowth and seed yield stability (Volenec *et al.,* 1996; Hirel *et al.,* 2007; Gaju *et al.,* 2014; Crews *et al.,* 2016). In perennials, N translocation from belowground organs and leaves is a key mechanism to meet periods of high demand (i.e. seed filling), but could compromise next-season regrowth and seed yield (Crews *et al.,* 2016). These tradeoffs between N allocation to reproduction or storage could be one of the causes of the compromise between yield, yield stability and longevity. Moreover, changes in N allocation might alter the source–sink ratio, which modulates how nitrogen is acquired and used for growth, seed-yield production and regrowth, controlling N remobilisation and storage within the leaf and the whole plant (Pornon & Lamaze, 2007; Wang *et al.,* 2016). In this sense, N allocation would regulate NUE (Table 1), both at leaf- and plant-level, and its components: N mean residence time (MRT; Table 1) and N productivity (AN; Berendse & Aerts, 1987).

Here we used as a model, two perennial species of *Physaria* and compared the pattern of N allocation, use and conservation of high-yield accessions (Y), and stable yield accessions (S), with their wild counterpart (W). Our general objective was to assess the effect of selection scheme for increased yield and sustained yield stability on the N economy of two *Physaria* perennial species. Our objectives were:

1. To evaluate the effect of selection scheme (high-yield or yield stability) on the pattern of N allocation. Changes in biomass allocation will be coupled to changes in N allocation. We predict that high-yield accessions (Y) will increase N allocation

to seeds at the expense of vegetative storage organs (leaf and root), while stable accessions (S) will show an allocation pattern similar to that of wild accessions (W).

2. To evaluate the effect of selection scheme on the pattern of nitrogen use and conservation. Changes in nitrogen acquisition capacity and NUE components will be coupled to changes in N source–sink ratio provoked by selection scheme. We predict that accessions Y will have a higher N acquisition rate, nitrogen productivity (AN), and N loss than W accessions while the MRT will be lower. The yield stability selection scheme will not change N acquisition capacity or NUE components. We predict that S accessions will maintain the conservative characteristics (lower N acquisition rate, lower N productivity, higher MRT and lower N loss) of W accessions.

3. To assess the existence of tradeoffs between seed-yield and N conservation capacity. Changes in seed yield will be related to changes in N conservation capacity. We predict that plant traits that enhance productivity (i.e. N acquisition rate, N allocation to seeds and nitrogen productivity) will be negatively related to those that promote N conservation (i.e. N allocation to vegetative organs and MRT).

Materials and methods

Plant species and selection schemes

The genus *Physaria* has been proposed as a potential new seed-oil crop for irrigated valleys in Patagonia (Argentina), because it contains high levels of hydroxy fatty acids similar to those of castor oil (lesquerolic, auricolic and densipolic acids; Thompson & Dierig, 1994; Dierig *et al.,* 1993); and because it exhibits several morphological, physiological and phenological traits that could contribute to increase ecological and economical sustainability in marginal environments (Ravetta & Soriano, 1998; Ploschuk *et al.,* 2001; González-Paleo & Ravetta, 2011a).

We used three accessions of *Physaria mendocina* and *Physaria pinetorum*: *wild* (*W*), *high-yield* (*Y*) and *stable* (*S*). These three accessions were selected by different schemes of selection (González-Paleo, 2010):

1. *Wild accession* (W): Germoplasm from seed-enhancement plots which were established from seeds collected from wild stands of each species.
2. *High-yield accession* (Y): Germoplasm collected from wild stands was selected by high seed yield for four

Table 1 Definition and units of traits used to describe N allocation, use and conservation traits at leaf- and plant-level

Variable	Definition	Units
Nitrogen allocation traits		
Npool	Plant total nitrogen content	mgN plant^{-1}
Nyield	Nitrogen content of plant seed yield	mgN total seed plant^{-1}
RNR	Root nitrogen ratio. Ratio between nitrogen in root and Npool	mgN in root mgN plant^{-1}
SNR	Stem nitrogen ratio. Ratio between nitrogen in the stem and Npool	mgN in stem mgN plant^{-1}
LNR	Leaves nitrogen ratio. Ratio between nitrogen in the leaves and Npool	mgN in leaves mgN plant^{-1}
SupportNR	Reproductive support structures nitrogen ratio. Ratio between nitrogen in the reproductive support structures and Npool	mgN in reproductive support structures mgN plant^{-1}
NHI	Nitrogen harvest index. Ratio between Nyield and Npool	mgN mgN plant^{-1}
Nitrogen use and conservation traits		
MRT$_{Plant}$	Mean residence time. Mean period during which N can be stored within the plant	year
Nloss	Total amount of nitrogen lost in senescent leaves, reproductive support structures and seed yield from an individual plant in a year.	mgN plant^{-1} year^{-1}
Nacq	Amount of nitrogen acquired by unit of root and time	gN g of root^{-1} year^{-1}
NUtE	Nitrogen utilisation efficiency. Yield produced per gram of Npool	g of seed gN^{-1}
AN$_{Plant}$	Nitrogen productivity. Dry matter production rate per unit of nitrogen stored within the plant	g biomass year^{-1} gN plant^{-1}
NUE$_{Plant}$	Nitrogen use efficiency. Biomass production per unit of nitrogen lost	g of plant gNloss^{-1}
AN$_{Leaf}$	Nitrogen productivity at the leaf level. Leaf dry matter production rate per unit of nitrogen stored within the leaves	g of leaves year^{-1} gN leaves^{-1}
MRT$_{Leaf}$	Mean residence time at the leaf level. Mean period during which nitrogen can be used for carbon fixation	year
NUE$_{Leaf}$	Nitrogen use efficiency at the leaf level	g of leaves gNloss by leaf senescence^{-1}

generations. The selection criterion was individual plant seed yield applied on stands of plants growing in introductory gardens in the Chubut River Valley (160 plants per species). The four most productive plants per generation were selected and seed from those plants was used to produce the next generation's stands.

3. *Yield stability accession* (S): Stable accessions (S) were obtained from plants selected by their low coefficient of variation in seed yield (CV$_{seed-yield}$) over time (CV$_{seed-yield}$ < 0.5) during three consecutive years. The CV$_{seed-yield}$ in *P. mendocina* was 0.43, with a CV$_{seed-yield}$ maximum of 1.43, while in *Ph. pinetorum* was 0.36, with a CV$_{seed-yield}$ maximum of 1.25.

The wild accessions of both species were the source of base germplasm to generate the selected accessions (Y and S) through mass selection (González-Paleo, 2010). There was only a Y and S accession per species. Introductory gardens where mass selection took place and the field plots of these experiments were located in the same location. Experimental conditions were as similar as possible. Seeds were sown mid-February. Seedlings were transplanted to the field 45 days after sowing (early April). Plant density was 16 plants m^{-2}, which is low enough to avoid detrimental effects of competition on final biomass, on the probability of flowering and on seed yield (Brahim *et al.,* 1998). Plots were flood irrigated every 20–25 days until

field capacity from September to April and weeds were removed manually.

In both experiments, accession Y of both perennial species showed higher biomass and seed yield ($F = 21.51$, d.f. = 2, 60; $P < 0.0001$; 32.3 ± 2.5 g of dry mass per plant; $F = 27.09$, d.f. = 2, 60; $P < 0.0001$; 11.5 ± 0.9 g seed dry mass per plant) than that of S (15.1 ± 1.6 g of dry mass per plant and 4.5 ± 0.6 g of seed dry weight per plant) and W accession (19.7 ± 3.4 g of dry mass per plant and 4.5 ± 0.5 of seed dry weight per plant). Stable and Wild accessions did not differ in biomass or seed yield.

Study site and experimental design

We carried out two completely randomised field experiments in 2013/14 and 2015/16 in the lower valley of the Chubut River, Patagonia, Argentina (43°17′, 65°29′). Both experiments had two factors: Species (two levels: *P. mendocina* and *Ph. pinetorum*) and selection scheme (three levels: no selection, W; high yield, Y and yield stability, S). The experimental unit was the plot (six plots per accession, species and year, for a total of six plots for each of the 12 treatments – three accessions, two species, 2 years). Seeds were sown mid-February in germination trays filled with soil, peat moss and sand in equal proportions and maintained in a greenhouse, where they received 80% of outside light levels and a temperature

range of 25–15°C (average daytime/nighttime temperature). Seedlings were transplanted to the field 45 days after sowing (early April). Plant density was 16 plants m^{-2}, arranged in rows 0.40 m apart and plants within a row were 0.17 m apart. Density was low enough to avoid detrimental effects of competition on final biomass, on the probability of flowering and on seed yield (Brahim *et al.*, 1998). Plots were flood irrigated every 20–25 days until field capacity from September to April. Weeds were removed manually.

This target area of cultivation is characterised by semi-desert Mediterranean conditions, with cold, wet winters and dry summers. Irrigation is possible from spring (September) to early fall (April). In this area, the mean annual precipitation is 179 mm, mean low temperature of the coldest month (June and July) is 1°C and absolute minimum air temperature is 0.8°C. The weather in year 2013/14 was wetter and hotter than in year 2015/16 (Fig. 1). In year 2015/16 mean precipitation was 107 mm, meaningfully lower than the mean annual precipitation, while in year 2013/14 with 184 mm it ranged between the mean values.

Chemical analysis, N allocation, use and conservation traits

We harvested one plant per plot for a total of six plants per accession, species and year (i.e. six plants per treatment) in the phenological stage of maturity, 330 days after sowing. Plants were oven-dried for 72 h at 50°C. Biomass was partitioned in roots, stem, senescent and green leaves, support structures and seeds. Seed yield was estimated as the product of the number of fruits, number of seeds per fruit and dry mass of a single seed. Nitrogen concentration

([N], mgN g^{-1} of dry biomass) was measured in roots ([N]roots), stem ([N]stem), green ([N]green) and senescent ([N]senescent) leaves, support reproductive structures ([N]support) and seeds ([N]seed) by the standard Kjeldahl acid digestion method (Scales & Harrison, 1920).

We separated traits in two groups (see Table 1 for definitions and units):

1. *Nitrogen allocation traits.* Using biomass and [N] data we calculated: Npool as whole-plant nitrogen content, Nyield as the seed nitrogen content, and proportional allocation of nitrogen to roots (RNR; root N ratio), stem (SNR; stem N ratio), leaves (LNR; leaves N ratio), support reproductive structures (SupportNR; support N ratio) and seeds (NHI; nitrogen harvest index). All data of nitrogen concentration described above were also included in this group.

2. *Nitrogen use and conservation traits.* We calculated: MRT and Nloss, as traits related to the conservation strategy. Nitrogen acquisition rate (Nacq), nitrogen utilisation efficiency (NUtE; Good *et al.*, 2004); N productivity (AN), and NUE (Berendse & Aerts, 1987; Hirose, 2012) were used as traits related to N use. NUE and its components were calculated both at leaf- (AN$_{Leaf}$, MRT$_{Leaf}$ and NUE$_{Leaf}$) and plant-level (AN$_{Plant}$, MRT$_{Plant}$ and NUE$_{Plant}$) because it has been proposed that there would be a strong relation between them (Hirose, 2012), thus, changes at leaf-level would affect plant-level nitrogen use and conservation. NUE is defined as the rate of dry matter production per unit of N lost in a determinate period of time (Berendse & Aerts, 1987; Vázquez de Aldana & Berendse, 1997). Here, this period was considered to be all the growth
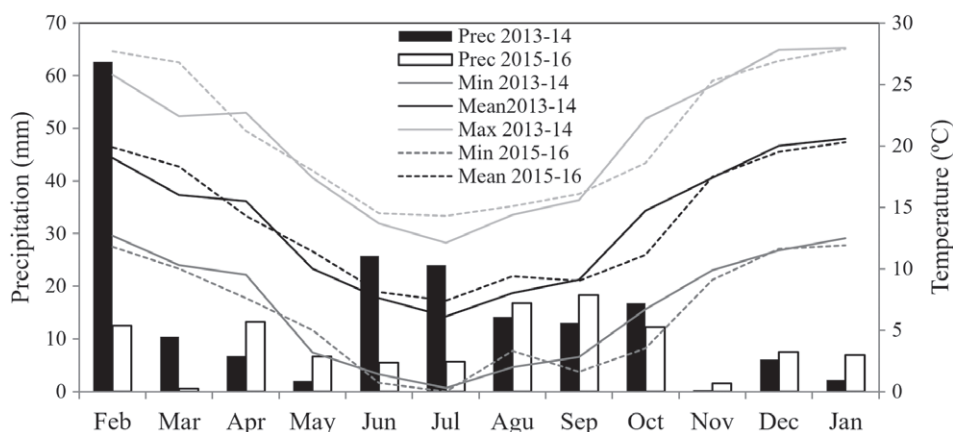

Figure 1 Pattern of monthly temperature and precipitation during both the experiments. Total precipitation was higher in the season 2013–14 than in 2015–16 (184.2 mm vs 107.5 mm), mean temperature was similar (13.60°C vs 13.85°C), although in October 2015–16 temperature was lower than in October 2013–14, coinciding with flowering period.

season from seed shown to harvest at maturity. In turn, NUE was the product of two variables: MRT and N productivity (AN). MRT is the inverse of the N requirements per unit of N in the plant (this parameter is known as Ln, and is the ratio between N loss and N content, thus: $1/Ln = MRT$) and AN is the rate of dry matter production per unit of N within the plant. We calculated AN as the ratio between dry matter production along the growth cycle and N content at the moment of the harvest (Yuan *et al.,* 2008). MRT was the ratio between N content at the moment of the harvest and N loss along the growth cycle. We used N content at the moment of the harvest as reference for these calculations because at this point, plants achieve their maximum biomass. Besides, the ways in which this N is partitioned, used and conserved would be relevant for plant fitness in the following season (Crews *et al.,* 2016).

Statistical analysis

A three-way analysis of variance (ANOVA) was carried out to determine differences in N allocation, use and conservation traits between accessions, species and years and their interactions (double interactions: year × species, accessions × species, species × year, accessions × year; and triple interaction: accessions × species × year). Standard error of the difference (SED) and least significant differences (LSD) were calculated at $P \leq 0.05$ in order to compare the means among accessions. We did not assess differences between species and years and interaction species × year because it was not the objective of this work, although we accounted for that variation in the ANOVA table in order to prove that the residual term in the ANOVA was based on the correct degrees of freedom.

To investigate tradeoffs between productivity (assessed as yield) and N conservation capacity (assessed as MRT at plant level), we carried out correlation test between traits related to productivity (seed yield, N acquisition, NHI, N loss, NUtE, AN_{Leaf} and AN_{Plant}; Berendse & Aerts, 1987; Good *et al.,* 2004; Dawson *et al.,* 2008) and traits related to N conservation (RNR, LNR, N loss and MRT_{Leaf}, MRT_{Plant}; Volenec *et al.,* 1996; Hirose, 2012; Crews *et al.,* 2016). Pearson's tests were carried out for variables meeting the normality assumption, whereas Spearman's tests were carried out for those variables in which normality assumption was not met (i.e. rank transformation for MRT_{Plant}).

To check for data normality we used Shapiro–Wilk's test and Levene's test for homogeneity of variance. A natural logarithm (for Npool, Nyield, RNR, LNR, seed yield, total biomass, Nacq, Nloss, AN_{Plant}, NUE_{Plant},

AN_{Leaf}, MRT_{Leaf} and NUE_{Leaf}) or rank (for MRT_{Plant}) transformation was applied when necessary to satisfy the assumptions of ANOVA. The use of rank transformation in parametric ANOVA proposed by Conover & Iman (1981) has been shown to produce reliable conclusions for main effects, although interactions are subjected to big increases in Type I errors (i.e. statistical significance where there is none; Salter & Fawcett, 1993; Higgins & Tashtoush, 1994; Wobbrock *et al.,* 2011). To avoid this problem we carried out an Aligned Rank Transform (ART; Wobbrock *et al.,* 2011) for MRT_{Plant} using the ARTool software in order to assure that the results of the interactions in the parametric ANOVA were reliable. Infostat 2009 was used for ANOVA and correlation analyses.

Results

Effect of selection scheme on N allocation, use and conservation traits

We compared traits related to N allocation, use and conservation among wild (W), high yield (Y) and stable (S) accessions of *P. mendocina* and *Ph. pinetorum* during 2 years. There was neither triple (species × accessions × year) nor double interaction (species × accessions; accessions × year), hence differences between accessions were consistent for both species and years (Table 2).

Y accessions showed a larger total Npool and total seed N content (Nyield) than W and S accessions (Table 3). The proportional allocation of N to seed (NHI) was higher in Y than in the others because of a higher seed production (data shown in Section 2), because there were no differences in N concentration among accessions ([N]seed; Table 3). Allocation to support reproductive structures was higher in W accessions (SupportNR and [N]support; Table 3).

Accessions Y showed lower N allocation to vegetative organs (RNR and LNR) and lower [N]roots and [N]leaves than W and S accessions (Table 3).

Y accessions had a higher N acquisition rate (Nacq) and were more efficient in N utilisation for seed production (NUtE) but they also showed a higher N loss and lower plant-level MRT (MRT_{Plant}) resulting in lower N use efficiency (NUE_{Plant}) than that of W and S accessions. There were no differences in N productivity (AN_{Plant}) among accessions. Stable accessions, in which the demand for N of reproductive sinks (NHI) did not increase as much as in Y accessions, maintained a similar pattern of use and conservation of N at plant-level than their wild counterparts. Nonetheless, at leaf-level, accessions Y and S showed lower MRT (MRT_{Leaf}) and N use efficiency (NUE_{Leaf}) than W accessions, while there were no significant differences in N productivity among accessions (AN_{Leaf}; Table 3).

Table 2 Sources of variation in N allocation and use and conservation traits

d.f. (total = 71)	Year 1	Sp 1	Accession 2	Year × Sp 1	Year × Accession 2	Sp × Accession 2	Year × Sp × Accession 2	MS_{error} 60
Nitrogen allocation traits								
Npool (mg $plant^{-1}$)	**36.91***	0.84[ns]	**22.49***	3.09[ns]	0.95[ns]	1.03[ns]	0.87[ns]	0.04
Nyield (mg $plant^{-1}$)	3.39[ns]	**13.89***	**26.49***	0.57[ns]	0.14[ns]	0.66[ns]	0.75[ns]	0.06
NHI (g g^{-1})	**34.67***	**42.42***	**15.76***	**19.31***	0.24[ns]	0.48[ns]	0.24[ns]	0.01
RNR (g g^{-1})	**9.52****	**5.47***	**34.10***	0.53[ns]	2.93[ns]	3.14[ns]	1.05[ns]	0.04
SNR (g g^{-1})	**12.41***	**13.29***	**20.28***	0.14[ns]	0.17[ns]	0.28[ns]	1.39[ns]	0.11
LNR (g g^{-1})	**14.46***	**19.35***	**11.82***	**30.04***	1.51[ns]	2.03[ns]	0.54[ns]	0.04
SupportNR (g g^{-1})	**8.43****	**18.53***	**5.62***	0.56[ns]	2.40[ns]	0.56[ns]	1.54[ns]	0.01
[N]seed (mg g^{-1})	**47.01***	**25.06***	1.23[ns]	2.05[ns]	0.24[ns]	0.20[ns]	3.40[ns]	0.002
[N]root (mg g^{-1})	**4.14***	**13.95***	**3.69***	0.40[ns]	0.69[ns]	1.40[ns]	0.10[ns]	0.01
[N]stem (mg g^{-1})	**5.00***	**38.04***	3.15[ns]	2.72[ns]	0.49[ns]	0.32[ns]	0.84[ns]	23.64
[N]leaves (mg g^{-1})	**4.17***	**7.63****	2.63[ns]	**22.49***	0.49[ns]	0.25[ns]	0.82[ns]	20.31
[N]support (mg g^{-1})	**52.18***	**23.17***	**3.89***	**18.71***	1.39[ns]	0.18[ns]	0.29[ns]	10.72
Nitrogen use and conservation								
Nacq (g g^{-1} $year^{-1}$)	0.22[ns]	**50.88***	**20.53***	0.70[ns]	0.17[ns]	0.06[ns]	1.38[ns]	0.02
NUtE (g g^{-1})	**73.21***	**16.94***	**15.63***	**12.14***	0.05[ns]	0.50[ns]	1.07[ns]	11.82
Nloss (mg $plant^{-1}$ $year^{-1}$)	**16.76***	3.43[ns]	**28.33***	0.21[ns]	0.96[ns]	1.08[ns]	1.18[ns]	0.04
AN_{Plant} (g g^{-1} $year^{-1}$)	**86.53***	1.33[ns]	2.64[ns]	**14.24***	3.38[ns]	0.94[ns]	1.93[ns]	0.003
MRT_{Plant} (year)	**28.42***	1.73[ns]	**18.39***	**33.02***	1.23[ns]	2.07[ns]	0.53[ns]	185.46
NUE_{Plant} (g g^{-1})	**12.46***	**8.42****	**4.56***	0.02[ns]	0.28[ns]	0.91[ns]	0.13[ns]	0.006
AN_{Leaf} (g g^{-1} $year^{-1}$)	**4.34***	**10.26****	1.33[ns]	**52.96***	1.81[ns]	2.45[ns]	0.30[ns]	0.01
MRT_{Leaf} (year)	**6.72***	1.96[ns]	**6.78****	**15.27***	0.91[ns]	1.09[ns]	1.84[ns]	0.14
NUE_{Leaf} (g g^{-1})	**5.38***	**7.18****	**7.39****	**4.55***	0.74[ns]	1.65[ns]	2.22[ns]	0.10

F-values and significance of main effects and interactions of year of experiment (Year), species (Sp) and selection scheme (Accession) in ANOVA analyses are shown ($n = 6$).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns, non-significant. Bold values indicate significant results.

Nitrogen allocation traits: Npool, total N in plant; Nyield, N in seed yield; NHI, N harvested index; RNR, root nitrogen ratio; SNR, stem nitrogen ratio; LNR, leaves nitrogen ratio; SupportNR, reproductive support structures + fruits N ratio.

Nitrogen use and conservation traits: Nacq, N acquisition rate; NUtE, N utilisation efficiency; Nloss, N loss rate; AN_{Plant}, plant-level N productivity; MRT_{Plant}, plant-level mean residence time; NUE_{Plant}, plant-level N use efficiency; AN_{Leaf}, leaf-level N productivity; MRT_{Leaf}, leaf-level mean residence time; NUE_{Leaf}, leaf-level N use efficiency.

Tradeoffs between yield and N conservation

Considering all accessions together, we found a negative relationship between seed yield and MRT_{Plant} (Table 4 and Fig. 2). Y accessions were located at the upper left portion of the figure, showing high seed yield and a low MRT_{Plant}. S and W accessions were scattered across the figure, showing high variability in both traits. This pattern was similar for all investigated tradeoffs (Figs 3 and 4). Specifically, for yield there were general tradeoffs with N stored in roots (Table 4 and Fig. 3A) and leaves (Table 4 and Fig. 3B) and with leaf-level conservation capacity (MRT_{Leaf}; Table 4 and Fig. 3C). On the other hand, we found tradeoffs between plant-level N conservation capacity (MRT_{Plant}) and N allocated to seeds (NHI; Table 4 and Fig. 4A), N loss (Table 4 and Fig. 4B), Nacq (Table 4 and Fig. 4C), NUtE (Table 4 and Fig. 4D) and leaf- and plant-level AN (Table 4 and Fig. 4E and Fig. 4F). In consequence, traits that enhance yield were negatively related to those that promote N conservation: N stored in roots (RNR) was negatively related to NHI, Nacq, Nloss, NUtE (Table 4). We

also found tradeoffs between allocation to leaves (LNR) and NHI, and AN (to leaf-AN_{Leaf} and plant-level-AN_{Plant}; Table 4). Leaf-level MRT (MRT_{Leaf}) was negatively related to Nacq, AN_{Leaf} and AN_{Plant} (Table 4).

Finally, we analysed tradeoffs separately for each accession. In general, all accessions present similar strength and pattern of correlation in the investigated tradeoffs than the general pattern (Figs 3 and 4).

Discussion

Effect of high-yield selection scheme

It has been shown that traditional breeding provoked, along with increase in seed yield and biomass, changes in C allocation such as a decrease in carbon storage and biomass allocation (Vilela & González-Paleo, 2015). Here we found that changes in N allocation provoked by high-yield selection resembled those in C allocation previously reported for *Physaria* species (Ploschuk *et al.,* 2005; González-Paleo & Ravetta, 2011a): Y accessions increased

Table 3 Mean comparisons between accessions (i.e. selection scheme; $n = 24$) for N allocation and use and conservation traits

| | Accession: Selection Scheme | | | | |
	High yield (Y)	Yield stability (S)	Wild (W)	SED	LSD$_{0.05, 60}$
Nitrogen allocation traits					
Npool (mg)	2.77 (652.34)	2.42 (304.32)	2.52 (396.32)	0.05	0.12
Nyield (mg)	2.56 (393.71)	2.09 (158.76)	2.13 (160.65)	0.07	0.14
NHI (g g^{-1})	0.63	0.52	0.45	0.03	0.06
RNR (g g^{-1})	−1.83 (0.016)	−1.39 (0.044)	−1.52 (0.032)	0.03	0.06
LNR (g g^{-1})	−1.04 (0.11)	−0.81 (0.19)	−0.80 (0.19)	0.06	0.12
SupportNR (g g^{-1})	0.24	0.22	0.31	0.03	0.06
[N] root (mg g^{-1})	1.19 (15.94)	1.26 (18.87)	1.25 (18.05)	0.03	0.06
[N] leaves (mg g^{-1})	21.91	24.37	24.60	1.30	2.60
[N] support (mg g^{-1})	10.12	10.40	12.52	0.95	1.89
Nitrogen use and conservation traits					
Nacq (g g^{-1} year^{-1})	0.08 (1.32)	−0.16 (0.78)	−0.17 (0.73)	0.04	0.08
NUtE (g g^{-1})	18.58	15.28	13.06	0.99	1.99
Nloss (mg plant^{-1})	−0.22 (582.80)	−0.66 (259.25)	−0.54 (338.92)	0.06	0.12
MRT$_{Plant}$ (year)	22.75 (0.97)	42.83 (1.17)	43.92 (1.13)	3.93	7.86
NUE$_{Plant}$ (g g^{-1})	1.75 (56.55)	1.78 (62.22)	1.83 (66.08)	0.02	0.05
MRT$_{Leaf}$ (year)	0.54 (5.07)	0.66 (6.17)	0.97 (16.39)	0.11	0.22
NUE$_{Leaf}$ (g g^{-1})	2.46 (402.32)	2.51 (384.45.48)	2.79 (977.14)	0.09	0.18

Only significant comparisons in analysis of variance (ANOVA) are shown. Standard error of the difference (SED) and least significant difference (LSD) for $P_{0.05}$ and 60 degrees of freedom are shown. Values in parenthesis are untransformed means.
Nitrogen allocation traits: Npool, total N in plant; Nyield, N in seed yield; NHI, N harvested Index; RNR, root nitrogen ratio; LNR, leaves nitrogen ratio; SupportNR, reproductive support structures + fruits N ratio.
Nitrogen use and conservation traits: Nacq, N acquisition rate; NUtE, N utilisation efficiency; Nloss, N loss rate; MRT$_{Plant}$, plant-level mean residence time; NUE$_{Plant}$, plant-level N use efficiency; MRT$_{Leaf}$, leaf-level mean residence time; NUE$_{Leaf}$, leaf-level N use efficiency.

Table 4 Pearson and Spearman's correlations between traits related to productivity and traits related to N conservation for all three accessions of both perennial species in the 2 years of experiment ($n = 72$)

| | Traits Related to Productivity | | | | | | |
	NHI (g g^{-1})	Nloss (g plant^{-1} year^{-1})	Nacq (g year^{-1} g^{-1})	NUtE (g g^{-1})	AN$_{Leaf}$ (g g^{-1} year^{-1})	AN$_{Plant}$ (g g^{-1} year^{-1})	Yield (g)
Traits related to N conservation							
RNR (g g^{-1})	**−0.36****	**−0.52*****	**−0.72*****	**−0.43*****	−0.20^{ns}	−0.19^{ns}	**−0.58*****
LNR (g g^{-1})	**−0.80*****	**−0.35****	−0.08^{ns}	**−0.73*****	**−0.81*****	**−0.34****	**−0.54*****
MRT$_{Leaf}$ (year)	**−0.48*****	−0.10^{ns}	**−0.31***	**−0.52*****	**−0.60*****	**−0.34***	**−0.29*****
MRT$_{Plant}$ (year)	**−0.75*****	**−0.30***	**−0.31***	**−0.76*****	**−0.75*****	**−0.46*****	**−0.51*****

Pearson's or Spearman's values are shown.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: non-significant. Bold values indicate significant correlations.
Traits related to productivity: NHI, nitrogen harvest index; Nloss, nitrogen loss rate including seed yield; Nacq, nitrogen acquisition rate; NUtE, nitrogen utilisation efficiency; AN$_{Plant}$, plant-level nitrogen productivity; AN$_{Leaf}$, leaf-level nitrogen productivity.
Traits related to N conservation: RNR, root nitrogen ratio; LNR, leaves nitrogen ratio; MRT$_{Plant}$, whole plant mean residence time; MRT$_{Leaf}$, leaf-level mean residence time.

their biomass and Npool, as well as their yield and N allocation to yield. As predicted, Y accession increased NHI at the expense of N allocation to vegetative storage organs. Given that nitrogen concentrations remained stable in seeds, the increase in Nyield is a mere consequence of higher production of seeds. The high nitrogen concentration of roots and leaves found in W accessions reinforce the idea proposed by Crews *et al.* (2016) that vegetative organs are high hierarchical sinks for both nitrogen and carbon in wild plants, while Y accessions favour reproductive sinks. These changes in the priority of sinks for N allocation could compromise regrowth in the next season (Volenec *et al.*, 1996; Crews *et al.*, 2016), altering plant longevity and inter-annual seed-yield stability. The increase in NHI found in Y accessions implies a decrease in N source–sink ratio which involves adjustments in acquisition capacity, foliar resorption and N loss as well as in the ways in which plants use N for growth or storage (Pornon & Lamaze, 2007; Marty *et al.*, 2010; Pornon *et al.*, 2011; Gaju *et al.*, 2014).

As predicted, we observed an increase in N acquisition (Nacq) per unit of root biomass during the first year
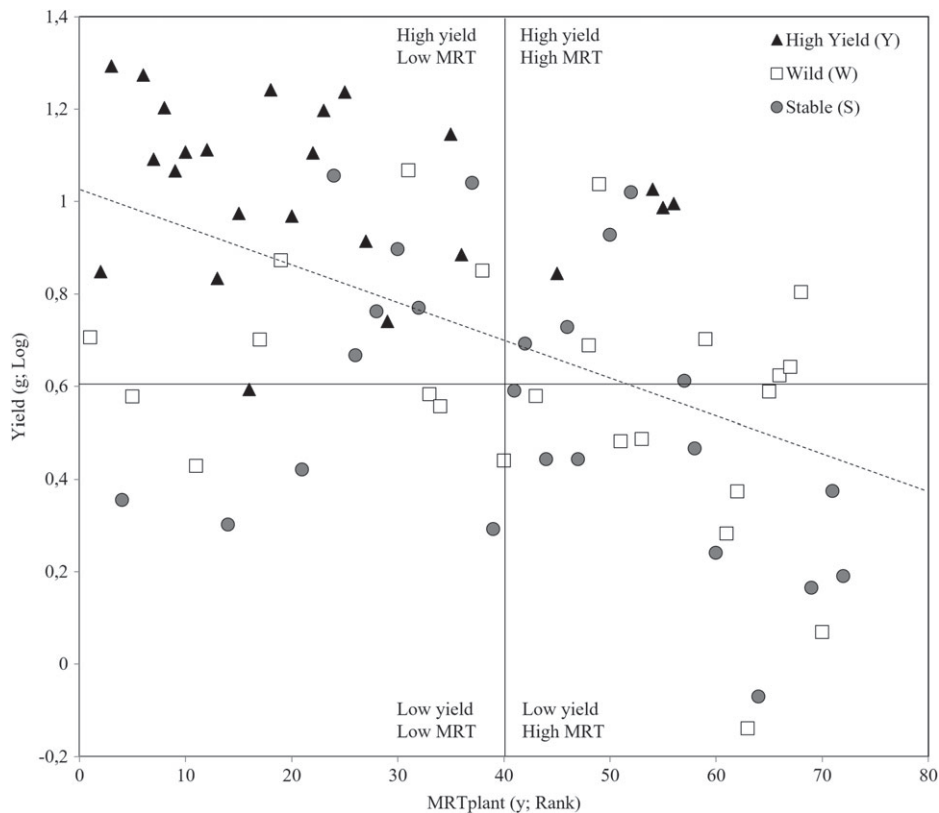
Figure 2 Correlation between mean residence time at plant level (MRT$_{Plant}$) and seed yield for high-yield accessions (Y), yield-stable accessions (S) and wild accessions (W) of two perennial species of *Physaria* in the 2 years of experiment ($n = 72$; $P < 0.0001$, Spearman's $R^2 = -0.51$). Seed yield and MRT data were transformed using natural logarithms and ranks, respectively, in order to fulfil with homoscedasticity and normality.
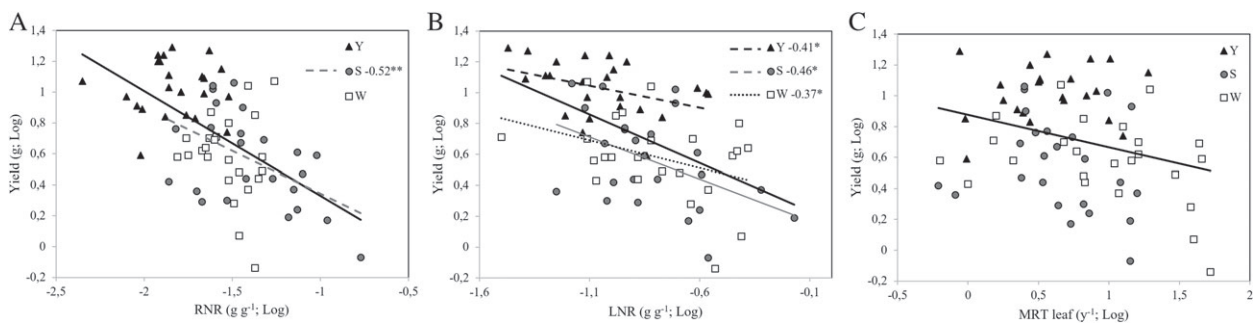

Figure 3 Seed-yield tradeoffs with N allocation and conservation traits: (A) yield versus root N ratio (RNR); (B) yield versus leaf N ratio (LNR); (C) yield versus leaf-level mean residence time (MRT$_{Leaf}$). Y are high-yield accessions (i.e. high yield selection scheme), S are yield-stable accessions (i.e. stable yield selection scheme) and W are wild accessions. Thick solid black line indicates a significant correlation for all accessions pooled together (coefficient and significance for these correlations are shown in Table 4). Correlations coefficient, significance (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) and direction for each accession are shown when significant.

of the crop. An increase in acquisition rate per gram of root might be a result of increases in specific root length (ratio between root length and root biomass; Reich *et al.,* 1998), which, in turn, may decrease the life span of roots (Eissenstat *et al.,* 2000) increasing N loss by root senescence (Silla & Escudero, 2004) and limiting N

storage capacity for next season regrowth (Volenec *et al.,* 1996).

This observed increase in NHI in Y accessions is associated to a higher N loss, both by seed harvest and by reducing N conservation at leaf-level and, in turn, a reduction of the overall N conservation capacity (MRT$_{Plant}$) and
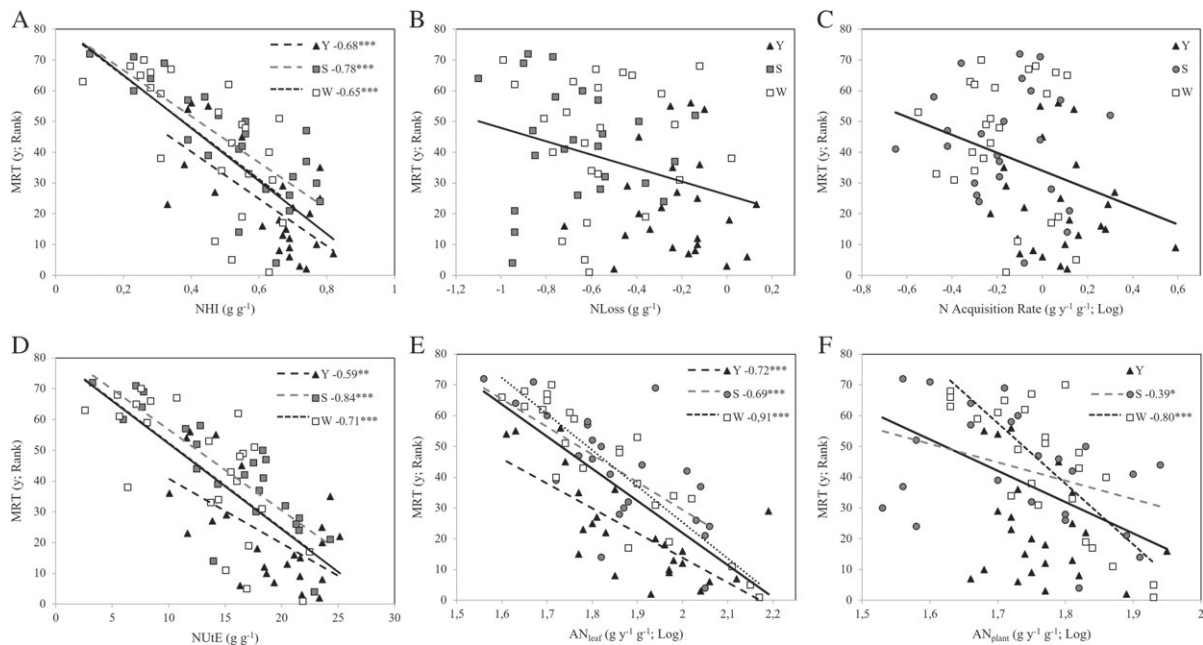
Figure 4 Nitrogen conservation capacity (MRT_{Plant}) tradeoffs with productivity related traits: (A) N harvest index (NHI); (B) Nloss; (C) N acquisition rate (Nacq); (D) N utilisation efficiency (NUtE); (E) leaf-level N productivity (AN_{Leaf}); (F) plant-level N productivity (AN_{Plant}). Y are high-yield accessions (i.e. high-yield selection scheme), S are yield-stable accessions (i.e. stable-yield selection scheme) and W are wild accessions. Thick solid black line indicates a significant correlation for all accessions pooled together (coefficient and significance for these correlations are shown in Table 4). Correlations coefficient, significance (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) and direction for each accession are shown when significant.

N use efficiency (NUE_{Plant}; Hirose, 2012). The decrease in the MRT at leaf-level found in Y accessions might be explained by changes in leaf physiology. Pornon *et al.* (2011) proposed that increase in the strength of reproductive sinks might alter nutrient resorption patterns and leaf life span. There are three possible mechanisms by which changes in leaf physiology might reduce nitrogen conservation capacity: (a) by increasing the N concentration in senescent leaves (i.e. lower resorption efficiency; García-Palacios *et al.*, 2013), (b) by increasing senescence rate (i.e. lower leaf life span; Berendse & Aerts, 1987) or (c) by using N resorbed from leaves for seed production at the expense of root or leaves storage (Crews *et al.*, 2016). All these changes would imply a higher Nloss, and lower MRT and NUE. The latter explanation seems to be the most consistent with our results, because the increase in N loss was related to lower LNR and RNR at the end of the first year, thus, decreasing N storage for the next season.

Finally, Y accessions were more efficient in N utilisation for seed yield production (NUtE) than W. Increased NUtE involve either a lower N investment in vegetative tissues or a higher N remobilisation from vegetative storage organs to seed production, leading to a lower invest of nitrogen per unit of seed produced (Andrews & Lea, 2013). Indeed, increases in NUtE in Y accession were

negatively related to N allocation to roots and leaves and to MRT (at leaf- and plant-level).

Effect of yield-stability selection scheme

Yield stability has been proposed to be one of the main advantages of perennial crop production (Glover, 2003). In fact, perennial crops need a sustained productivity for at least a few years after establishment to be competitive with their annual counterparts (Vico *et al.*, 2016). Therefore, it is important to check that increased yield does not affect N conservation capacity of plants. We showed that the allocation pattern of S accessions was very similar to that of W, except for an increase of their NHI at the expense of support reproductive structures. S accessions also kept the N use and conservation traits of their wild counterparts: low N acquisition rate and high dependence of internal N cycling (low Nloss and high MRT and NUE at plant-level). At leaf-level the N conservation mechanism of S accessions differed from that of wild plants, showing the former lower MRT_{Leaf} and NUE_{Leaf} than the latter. These could be related to the increase in the reproductive sinks strength of S accessions (NHI; Pornon *et al.*, 2011). However, these changes seem to have no consequences in the overall N conservation capacity of S accessions: roots and leaves are still the main destination of the reabsorbed

N in opposition to Y accessions, which favoured seeds production, hence, increasing Nloss and reducing the overall capacity for N conservation. Anyhow, leaf physiological and structural changes seem to play a relevant role in N conservation (Hirose, 2012), and more attention has to be paid to them in future perennial crop research.

Tradeoff MRT yield: implications on crop domestication

Indirect changes incurred during early stages of domestication of perennial species of *Physaria* are at odds with those required to develop a stable crop for semi-arid, Mediterranean environments (González-Paleo & Ravetta, 2011a). Our results support the existence of tradeoffs between seed yield and N conservation (measured as MRT at plant-level). Tradeoffs between seed production, allocation of N to storage organs and next year's growth often described for perennials (Crews *et al.,* 2016; Vico *et al.,* 2016) imply that species coming from low resource environments (such as W and S accessions) are less productive than those growing in fertile environments (i.e. Y accessions) but more efficient in N conservation, less dependent of external nutrient supply and more resilient to environmental variability (Vázquez de Aldana & Berendse, 1997; Aerts & Chapin, 2000; Silla & Escudero, 2004).

N invested in seeds account for more than a 65% of the total N lost in Y accessions (while in W and S it only represents 40% and 55% of the budget, respectively), at the expense of a reduction of N stored in root and leaves. In the light of these observations, it seems that N translocation from vegetative organs to reproductive machinery (seeds and support reproductive structures) is the main route for N loss in the reproductive stage of the Y plants. In addition, a lower MRT_{Leaf} might be indicating higher N loss by leaf senescence. These mechanisms involve a reduction in N storage and conservation that would lead to a higher reliance on external inputs and affect following year's regrowth, thus, reducing yield stability and life span (Jackson & Koch, 1997; Malézieux, 2012; Pimentel *et al.,* 2012; Crews *et al.,* 2016). In low N environments, plants with lower N conservation capacity, such as Y accessions, will produce more biomass and yield in the short term than S or W, but will have lower productive potential in subsequent seasons compared to species adapted to low N environments which have a longer MRT and lower rates of Nloss (Aerts & van der Peijl, 1993).

S accessions also increased their proportional Nloss by seed production compared to W, although they maintained similar patterns of N conservation, remaining in an intermediate position between the Y and W accessions allocation and conservation pattern. It has been proposed that a sustainable crop idiotype for low resource environments would be intermediate between wild plants and crop varieties (Dawson *et al.,* 2008). Our results indicate that a selection scheme for yield stability might be developed in order to accomplish with the sustainable crop idiotype for low resource environments. Moreover, these results indicate that there might be different options to increase nitrogen conservation in perennial oilseed crops. For example, selecting for high [N] leaves along with high yield might allow sustaining photosynthesis longer in the season, thus creating higher C and N storage for next season. Additionally, N stored in leaves might be remobilised if necessary, providing an important mechanism for maintaining productivity in perennial systems under variable environmental conditions (Crews *et al.,* 2016).

Concluding remarks

The desirable increases of biomass and yield as a result of selection for high yield are accompanied by changes in nitrogen allocation and NUE at leaf- and plant-level that might affect the ecosystem services provided by the perennial. Observed changes in allocation, nitrogen acquisition and use economy imply higher losses of nitrogen in the crop system and might be responsible for decreased longevity. The shift of strategy provoked by selection implies a decrease in NUE and conservation capacity that may be behind the loss of yield on subsequent years (Aerts & van der Peijl, 1993). Wild and stable accessions were more conservative and show traits at the leaf- and root-level that promote nitrogen conservation. These are key traits that should not be lost during the selection process if breeders want to achieve the adequate ideotype of perennial crop for arid systems.

Acknowledgements

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