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Domestication effects on nitrogen allocation, internal recycling and nitrogen use efficiency in the perennial new crop *Silphium integrifolium* (Asteraceae)

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Abstract

Improvements in seed yield during domestication and breeding are frequently achieved moving plants from the conservative syndrome of the plant economic spectrum towards the more acquisitive side, changing how plants acquire, allocate, use and store C and nutrients in relation to their wild relatives. The aims were to evaluate if domestication changed the N allocation, the internal plant N recycling (N resorption efficiency and proficiency, and N storage) and N-use-efficiency, in the perennial new crop *Silphium integrifolium*. We compared in a field experiment repeated in two locations (Kansas and Patagonia), a Wild (W) and two improved accessions, with high-(HYI) and low-seed yield improved (LYI) accessions. HYI accessions produced more than twice the biomass and acquired twofold more N than LYI and Wild accessions. Changes in proportional N allocation were similar in both improved accessions (HYI and LYI) and locations: higher allocation to leaves and lower to the crown at pre-anthesis (growth-storage trade-off) and higher allocation to seeds and lower to the crown at maturity (reproduction-storage trade-off). HYI and LYI allocated 50% more N to seeds in average, reducing a 31% the N allocated to the crown in relation to the Wild. Nitrogen use efficiency (NUE) and mean residence time (MRT) of N were reduced in both improved accessions. In HYI, the lower MRT was related to the lower storage of N in the crown and N removal through seed production, and in LYI was the result of the N lost through leaf senescence. HYI produced litter with lower N concentration (more proficient) that the wild accession. These could decrease the net N mineralization rate and soil N availability at long term. HYI plants should require higher external N inputs to the soil to sustain their N uptake requirements. LYI and Wild accessions, had lower resorption efficiency, but N recycled was allocated to the crown for future remobilization and uses (better internal cycling). The leaf litter type of these accessions with higher N content should sustain faster N-cycles rates and N release to the soil. If the focus is on the ecosystem services, HYI accessions could help achieve a reduction in nitrate leaching by maximizing N uptake, N resorption and N removal through seed (i.e. nitrate catch crop), and also produce crop residues that slow down biogeochemical cycling and soil nutrient retention.

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1 | INTRODUCTION

The main goal of agricultural research has been to increase crop productivity, however, there is also a strong push to maintain soil fertility and mitigate environmental pollution caused by the use of nutrient fertilizers required to increase yields. Several strategies have been identified that could reduce the amount of nitrogen fertilizer needed by agriculture, including a better management of the type, amount and timing of fertilizer application (Cassman et al., 2002); greater use of legumes that add N into agricultural systems via symbiotic nitrogen fixation in a sustainable manner (Biswas & Gresshoff, 2014); selection and breeding of plant cultivars that capture fertilizer and convert it to food and feed protein more efficiently (i.e., greater nitrogen use efficiency-NUE; Xu et al., 2012); among others. Maximizing the efficiency of resource use is becoming a highly prioritized issue in agricultural research. For example, there is increasing evidence that perennial cropping systems can utilize many resources more efficiently than annual systems (Jordan et al., 2007; Gonzalez-Paleo et al., 2019; Rajnoch et al., 2020).

In particular, de novo or direct domestication of perennial species into grain crops has been proposed as one approach to reduce or reverse negative environmental impacts of large-scale agriculture (Wagoner & Schaeffer, 1990). While perennial options currently exist for some crop niches, such as fruits or pastures, there are very few perennial alternatives to the annual cereal and oilseed grain crops that form the modern food systems. Among these new domesticates are intermediate wheatgrass (*Thinopyrum intermedium*), which produces a grain similar to wheat, perennial barley (*Hordeum* spp.), and silflower (*Silphium integrifolium*), a member of the sunflower family under development as an oilseed crop (Crews et al., 2018; DeHaan & Van Tassel, 2014; Glover et al., 2007; Marquardt et al., 2016).

Particularly for perennial plants, seasonal nitrogen recycling through the optimization of plant senescence and nitrogen resorption, is a determinant of N-use efficiency and N conservation (Millard & Proe, 1993), and it is also a determinant of plant fitness (i.e. survival, growth capacity and reproductive output; May et al., 1992), vital components of the perennial life-cycle (Staswick, 1994). The lack of remobilization of nutrients during senescence into storage organs, such as roots or crowns, could compromise re-growth of shoots the following growing seasons, and ultimately, the persistence of the perennial stand (Pastor-Pastor et al., 2018).

The ability to store and internally redistribute nitrogen resources is a fundamental element of the N economy of perennial plants (Aerts & Chapin, 2000; Killingbeck, 1996). The internal redistribution or recycling of N can be divided into four phases: (1) primary allocation of N from N-assimilating source tissues to sink tissues during the growing season; (2) reallocation of N resources arising from metabolic recycling of N during the growing season; (3) resorption of N from senescing tissues and its transport to seed filling during the reproductive phase or to perennating tissues during autumn senescence; and (4) remobilization of N from perennating tissues to actively growing tissues during spring flush. The last two phases constitute seasonal N recycling.

Nitrogen resorption from senescing leaves is usually considered an important component of N conservation, and it measure the amount of nutrients reallocated to storage organs (Eckstein & Karlsson, 1999). Storage organs are involved in plant survival and reproduction and are represented by tubers, bulbs, roots, rhizomes, crowns, seeds and fruits (Masclaux et al., 2001). For example, in annual crops NUE and resorption are evaluated considering seeds as storage organs for reproduction, which in an agricultural context often represent the major harvest product (Spiertz & De Vos, 1983) and therefore, the cause of N lost from the agroecosystem during harvest. In perennial crops, in addition to seed filling, a proportion of the nutrients remobilized from leaves is allocated to underground structures such as roots, rhizomes and crowns (Lemus et al., 2008; Nassi o Di Nasso et al., 2013), where they serve as nutritional reserves for regrowth in the next season. In an agricultural context, nutrient remobilization to underground organs is important for the sustainability of biomass and seed production in perennial crops (Gonzalez-Paleo et al., 2019; Pastor-Pastor et al., 2018).

A substantial proportion of nutrients remains in leaves after senescence. Resorption can not only be measured as the proportion of nitrogen resorbed from senesced leaves (resorption efficiency or NRE) but also as the nitrogen concentration in senesced leaves, called resorption proficiency by Killingbeck (1996). This author considered that plants with concentrations in senesced leaves lower than $7 \, \text{mg} \, \text{g}^{-1}$ are highly proficient. An important feature of knowing the levels to which nutrients can be reduced in senescing leaves, is that these values offer an objective method to quantify the success of resorption as a nutrient conservation mechanism. Also, proficiency is strongly associated with the quality of plant litter (Aerts & Chapin, 2000). Consequently, resorption not only influences plant growth, survival and yield stability, but also soil N availability and, thereby, ecosystem nutrient cycling (Killingbeck, 1996; Yuan & Chen, 2009).

Perennial plants have inherent N-conserving strategies, such as high N resorption (Heckathorn & Delucia, 1996), long-lived leaves (Gonzalez-Paleo et al., 2019; Rajnoch et al., 2020) and high N-use efficiency, as they are highly productive for each unit of N uptake (Pastor-Pastor et al., 2019). However, improvements in seed yield during the domestication and breeding are frequently achieved sacrificing some of these attributes of high ecological value (Denison, 2012; Van Tassel et al., 2010; Vilela & Gonzalez-Paleo, 2015) as they change the way that domesticated perennials acquire, allocate, use and recycle nitrogen in relation to their wild relatives (Pastor-Pastor et al., 2021).

For example, high-yielding selected accessions of perennial species of *Physaria* (Brassicaceae) showed higher N acquisition rate by roots, higher N allocation to seed at the expense of vegetative storage, and a lower N conservation and NUE in relation to wild accessions (Pastor-Pastor et al., 2018).

Understanding drivers of N conservation and N resorption in perennial crops might be a key issue to define ideotypes that minimize tradeoffs between yield and N-recycling. Here, we addressed the following questions: (1) Has N allocation to vegetative and reproductive structures changed during the domestication process of the perennial *Silphium integrifolium*? (2) Have internal plant N recycling (N resorption efficiency and proficiency and N storage) and N-use-efficiency changed during *Silphium* domestication? We compared, in a field experiment repeated in two locations, a Wild (W) and two improved accessions of *Silphium integrifolium*, with high- (HYI) and low-seed yield improved (LYI) accessions. We predict that (1) HYI accession will have a higher total N pool and will prioritize the allocation of N to reproductive structures at the expense of allocation to storage and vegetative organs (crown and leaves), in relation to Wild and LYI accessions; (2) HYI accession will exhibit a higher N resorption efficiency and a higher proficiency, but, overall higher N loss through leaf senescence (higher amount of senescent leaves), higher N removal through the harvest of seeds and lower N-use-efficiency, in relation to Wild and LYI accessions; and (3) N resorbed in HYI accession will be allocated mainly to seeds at the expense of less N recycling to the crown, in relation to LYI and Wild accessions.

We discuss the implications of our results in the context of yield stability in time and the ecosystem services provided through plant N seasonal cycling as a conservative strategy of wild perennials.

2 | MATERIALS AND METHODS

2.1 | *Silphium* accessions and experimental design

Field measurements were performed in the two locations described below, comparing nitrogen allocation, N conservation and N use efficiency of three accessions of *Silphium integrifolium*, during the second growth period of the crop: April–September 2017 in USA, and September 2017–April 2018 in Argentina.

Research plots were located at The Land Institute near Salina, Kansas, USA (38°46′14″N; 97°35′30″W) and at the Chubut River Valley (43°18′20″S; 65°19′15″W) near Trelew, Patagonia, Argentina. Locations' climate and experimental details are presented as supporting information. Both locations represent target environments where ecophysiology and agronomy of *Silphium* accessions are being evaluated to define a sustainable perennial crop ideotype (Vilela et al., 2018).

The breeding lineages at each location are quite similar (they are half-sibs), although not identical (not clones). Plants used in Patagonia are a five half-sib families' sub-sample of the 10 selected in Kansas. All of them came from a breeding population of half-sib open pollinated under recurrent selection for an increased number of ray florets per head as a proxy for seed yield (ray florets are female and seed

fertile; disk florets are male; Van Tassel et al., 2017) and for general vigour and plant health. This population had undergone seven cycles of selection, the selection protocol was described in detail in Vilela et al. (2018).

The breeding nurseries were established in the field in late May (spring) 2015 in Salina and December (spring) 2015 in Trelew, by transplanting approximately 1500, 8-week-old seedlings (300 plants of each five half-sibs families in Patagonia and 150 plants of each 10 half-sibs families in Kansas), in a completely randomized design (Vilela et al., 2018). The experimental design consisted in six plots of 50 plants for each half-sib family in Patagonia and 25 plants in Kansas (for a total of 30 and 60 plots in Patagonia and Kansas, respectively).

In both locations, all these plants were evaluated for seed yield in the previous years. Based on these data, in both locations, we selected the highest-yielding half-sib family and the lowest-yielding half-sib family from the breeding nursery. From these selected half-sib families, we also selected the six plants highest yielding (one plant per each plot, repetition; HYI) and six plants were selected based on the lowest seed yield (one plant per plot; LYI). In each location, plants of the half-sibs with high- or low-seed yield were considered as accessions HYI and LYI, respectively (Table 1). A population of 20 individual plants of Wild genotypes (W) from nearby germplasm evaluation plots were used for comparison; we measured traits on 6 individual plants of different plots (*n*). These plots were of a similar age and managed similarly to the breeding nursery experimental plot. The seed used to establish these plots was collected from wild native populations in Kansas.

Intra-accession differences in biomass and seed yield are due to the high incidence of pathogens (i.e., *Puccina silfii*) and insect pests (i.e., *Eucosma giganteana*) in Kansas that are not present in Patagonia (Turner et al., 2018; Vilela et al., 2018, 2020).

In both field experiments, plants in plots were arranged in rows set 1 m apart and plants spaced at 1 m within the row (1 m × 1 m). In Salina, transplants received additional water at field capacity at the time of transplanting, and no additional irrigation afterwards, the plants receiving only rainfall. In Trelew, 2 additional irrigations were applied during the reproductive period, taking the soil to field capacity, only during the first year of the growth cycle. While both Kansas and Patagonia environments have cold winters and hot summers, Patagonian valleys require irrigation for crop production, since precipitation is below 200 mm yr^{-1}. After the first year, *Silphium's* deep-root system appears to tap into the water table located between 1.5 and 2 m deep, allowing for reduced irrigation. Weeds were controlled using mechanical tillage and hand pulling, as necessary during the first growing season. No fertilizer was applied because our objective was to compare the internal N recycling strategy and N use efficiency between improved (HYI and LYI) and Wild accessions without external inputs.

2.2 | N allocation, recycling and use efficiency

In each location, we harvested plants at two stages of growth, preanthesis and maturity. At each stage, we recorded the total number of

TABLE 1 Seed yield and number of capitula per plant for the three groups of plants and both locations used in the experiment.

Locations	Yield-related traits	Accessions		
		HYI	LYI	Wild
Salina-Ks	Total biomass (g)	598 ± 60	273 ± 69	165 ± 36
	Seed yield (g pl^{-1})[a]	56.2 ± 4.8	28.6 ± 7.11	29.6 ± 4.1
	Capitula per plant	89.4 ± 4.7	44.0 ± 4.1	257.6 ± 18.6
Trelew-Pat	Total biomass (g)	1430 ± 185	847 ± 113	659 ± 99
	Seed yield (g pl^{-1})	197.8 ± 14.23	54.6 ± 3.3	50.8 ± 3.5
	Capitula per plant	167.8 ± 5.8	80.8 ± 9.2	218.6 ± 8.3

Note: $F_{biomass} = 14.3^{**}$; $F_{seed\ yield} = 11.44^{***}$; $F_{capitula} = 11.86^{***}$. Values are means ± SE, n = 6 (one plant per plot of the half-sib family with the highest and the lowest seed yield, for HYI and LYI, respectively; One plant per plot for Wild accession). The lower number of capitula per plant in improved accessions (HYI and LYI) in relation to Wild in both locations, is a consequence of domestication and breeding for high seed yield (decrease in capitula number and increase in seed number and seed size; Vilela et al., 2018).

Abbreviations: HYI, high yield improved; LYI, low yield improved; W, wild.

[a]The productivity difference between locations is due to the high incidence of several pathogens and insect pests in Kansas and no detectable herbivory or diseases in Patagonia (Vilela et al., 2020).

stalks and harvested two of them (with the corresponding portion of the crown where carbohydrates and N are stored in *Silphium*) from six plants per half-sib family (one rep per plot), and six plants of the Wild accession. Since the growth cycle in Trelew is longer than in Salina, we used the phenological stage of plants as criteria to determine the timing of harvest (Vilela et al., 2020). The two phenological stages in which we harvested the plants were: stage 3, pre-anthesis, when 50% of the plants had visible buds, without visible ray florets; and stage 7, mature capitula, when 50% of the plants had capitula with brown seeds. We selected these phenological stages because in pre-anthesis N green concentration reach the maximum values for *Silphium*, while in maturity this value is minimal indicating the maximum capacity of N resorption.

Plants were oven-dried for 72 h at 50°C. At pre-anthesis, the harvested biomass was partitioned into crown (it included the crown portion and the roots associated with it), stalks and green leaves. At maturity, plants were completely senesced, and biomass was partitioned into crown (crown + roots), stalks, senescent leaves, reproductive support structures (i.e., receptacle and involucre) and seeds. Nitrogen concentration ([N], mgN g^{-1} of dry biomass) was determined in crown (including a portion of roots), stalks, green and senescent leaves, support reproductive structures, and seeds by the standard Kjeldahl acid digestion method (Scales & Harrison, 1920).

Modular growth is the process whereby architectural units are replicated within a plant. Particularly, *Silphium* sp. produces several modules or stalks from the bud bank in the crown, as part of the normal development of the plant.

The cluster of stalks is replaced annually. This growth mechanism allows to maintain crown productivity and prolongs plant longevity (Valladares & Niinemets, 2007). The stalk is a very convenient unit of sampling because it is large enough to integrate all the most relevant physiological processes. For this reason, stalks can be used to scale up from the stalk-level to the whole plant level (Gartner, 1995).

Considering this, and using the measurements at stalk-level such as the biomass of one stalk, the proportional allocation to each organ per stalk, and [N] data, we calculated the whole plant traits using the total number of stalks per plant. We calculated the total N pool as whole-plant nitrogen content, and proportional allocation of nitrogen to the crown (CrownNR; crown N ratio, including a portion of roots), stalks (StalkNR; stalks N ratio), leaves (LeafNR; leaves N ratio), reproductive support structures (SuppNR; support N ratio) and seeds (SeedNR, Seed N ratio). The N content stored in the crown (PoolNCrown) at pre-anthesis and maturity was calculated by multiplying the [N] in the crown by the total crown biomass.

At maturity, we calculated traits related to: (1) N remobilization and recycling: proficiency, resorption efficiency, N_{veg} loss and N_{seed} loss; and (2) N use efficiency: N productivity (NP) and mean residence time (MRT). We used the oldest senescent leaves that were collected directly of the plants rather than from leaf litter to measure nitrogen concentration, as we wanted to avoid litter decomposition and leaching of leaf nutrients that would lead to underestimation of nutrient concentrations in senesced leaves. Due to the 'sequential' leaf senescence (i.e., a senescence progression with an acropetal gradient along the axis of the plant), we collected the bottom-leaves. A leaf was considered senescent when 50% of the leaf lamina had a yellow-brown colour.

Also, a leaf that is not completely senesced would lead to overestimate N concentration in senescent leaves (Gonzalez-Paleo et al., 2022). This procedure guarantees a complete resorption of N, and an adequate estimation of proficiency and resorption (Yuan et al., 2008).

Nitrogen resorption was estimated as proficiency (([N] senescent^{-1}) the minimum level to which a plant can reduce an element; nitrogen in this case), and efficiency (NRE, %, [([N]green–[N] senescent) /[N]green] *100; Killingbeck, 1996) the proportion of nitrogen resorbed during senescence. The simplest estimation of nutrient remobilization can be calculated through the 'apparent
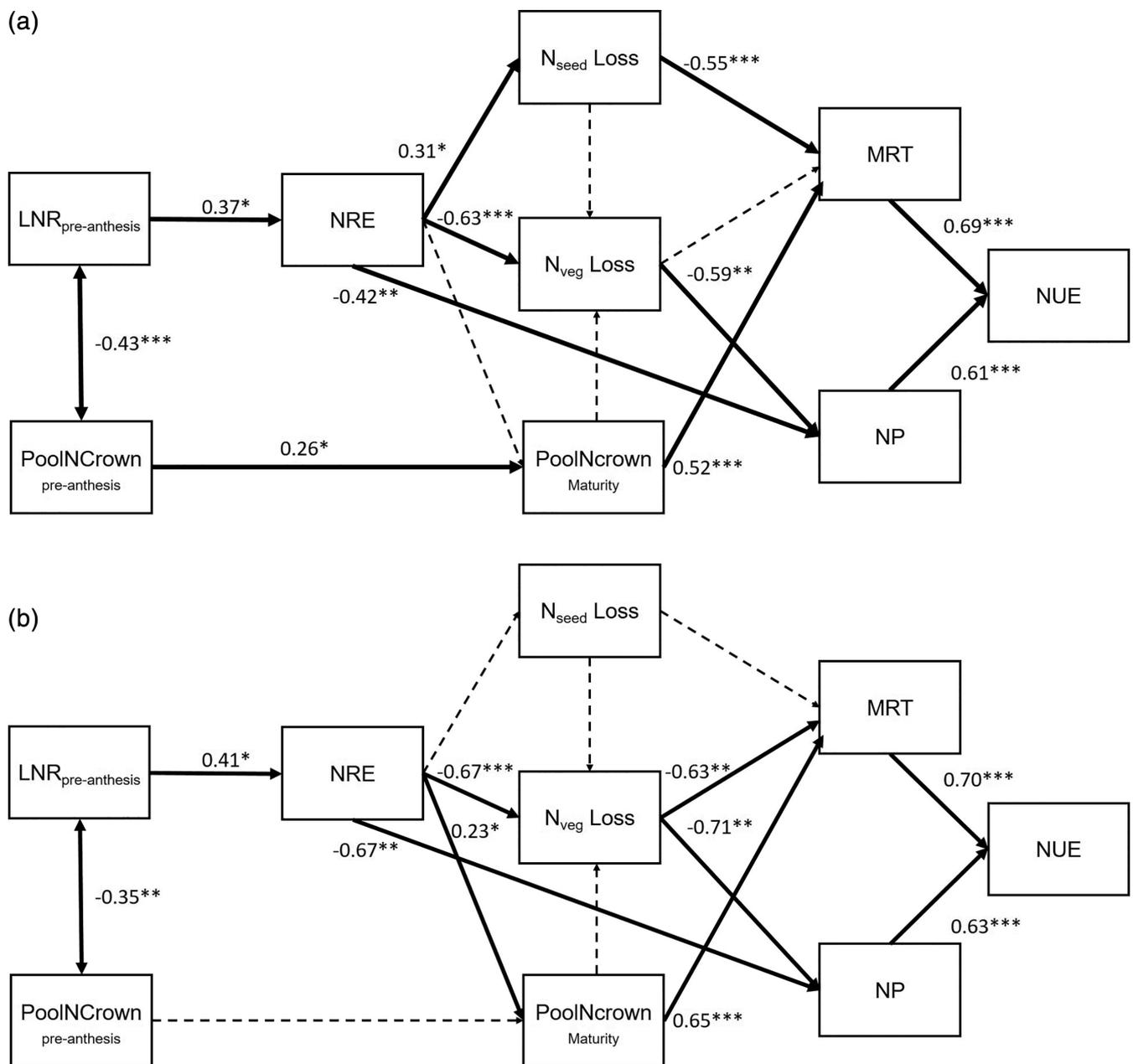
FIGURE 1 Path diagram showing the functional relationships between N allocation and N recycling traits and NUE and their components (NP and MRT) for (a) high yield improved and (b) low yield improved, compared with the Wild unselected accession. Each path analysis was performed with $n = 24$ (12 individual plants per accession in both locations, 1 plant per plot). Asterisks indicate significance of the coefficient in each path: ***$p < .001$; **$p < .001$; *$p < .05$. Numbers indicate the direct β path coefficient of the functional relationship between traits. The descriptive fit indices were CFI = 0.74 and NFI = 0.76, indicating a good fit. LNRpre-anthesis, proportional N leaf allocation; MRT, mean residence time; NRE, nitrogen resorption efficiency; N_{veg} loss, N lost from the plant due to leaf senescence; N_{seed} loss, N lost from the plant due to harvest; NP, nitrogen Productivity; NUE, N use efficiency; PoolNCrown, the N content stored in the crown at pre-anthesis and maturity.

remobilization' method, which relies on the determination of the amount of total nutrient present in the different plant organs at different times of development (before and after leaf senescence) as previously used by Hocking et al. (1977). The N loss from the plant was characterized by: N_{veg} loss as the N lost through leaf senescence; and N_{seed} loss as the N lost through seed production. N_{seed} loss is a variable that also represents the N removal from the ecosystem through harvest.

Nitrogen productivity (NP, g mg^{-1} weeks^{-1}; total dry mass*[Total N Pool*Time]$^{-1}$), mean residence time (MRT, weeks; [Total N Pool]* [((PoolNsenescent + Nyield)*time)$^{-1}$]), and nitrogen use efficiency (NUE, g g^{-1}; MRT*NP) at the plant level were calculated following the models proposed by Yuan et al. (2008). Nitrogen utilization efficiency (NUtE) was calculated as a way to characterize the accessions ability to produce seeds per unit of N taken up.
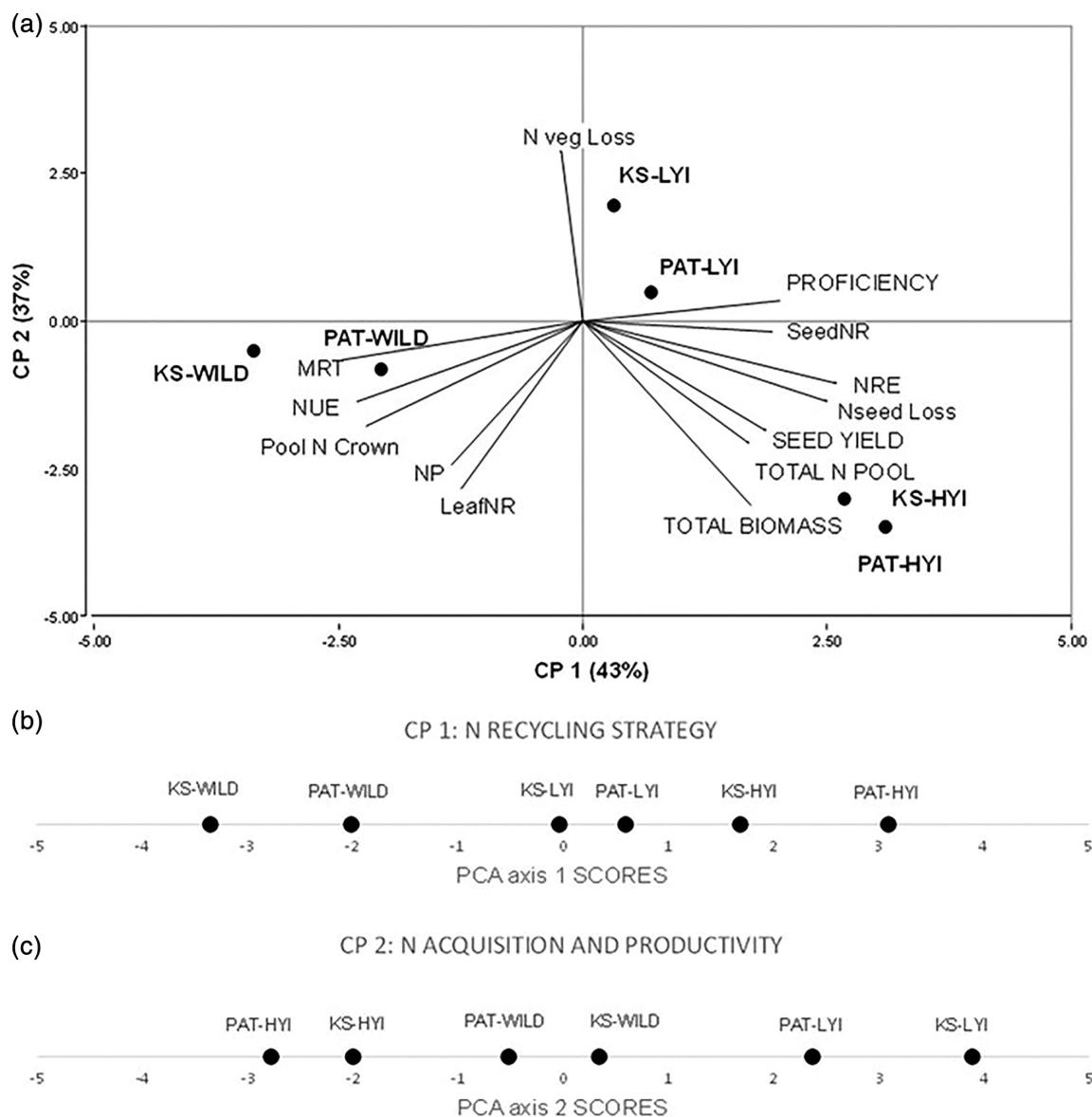
FIGURE 2 (a) A biplot of three accessions (Wild; LYI, low yield improved; HYI, high yield improved) of *Silphium* growing at two locations (KS, Kansas; PAT, Patagonia) based on productive traits (total biomass and seed yield) and N allocation and recycling traits. The first two principal components, principal component 1 (PC1) and principal component 2 (PC2) are plotted, each accounting for a proportion of the variance in the original dataset, shown in parentheses. Accessions in each location are represented by points and traits by vectors. Each point in the bipot is an average value of 6 plants per accession per location (*n*). Accessions in each location are plotted according to their scores in the PCA axis 1 related to 'N recycling strategy' (B) and PCA axis 2 related to 'N acquisition and productivity' (C).

2.3 | Statistical analysis

For each phenological stage, a two factors ANOVA was performed to compare the N allocation, N conservation and N use efficiency between accessions (three levels: HYI; LYI and Wild) and locations (Patagonia and Kansas). Non-interaction between Accessions and Locations were detected for any trait, thus we test differences between main factor (accessions and locations) in the two Way-ANOVA. Standard error of the difference (SED) and least significant differences (LSD) were calculated at *p* ≤ .05 in order to compare the means among accessions. To check for data normality

we used Shapiro–Wilk's test and Levene's test for homogeneity of variance. A natural logarithm (for Total N pool, N_{rep} Loss, MRT, PoolNcrown, NUE and NUtE) or square root (CrownNR and LeafNR) transformation was applied when necessary to satisfy the assumptions of ANOVA.

A principal component analysis (PCA) was used to explore the distribution of accessions (HYI, LYI and W) in each location (Pat-Patagonia, and KS, Kansas) relative to their productivity and N cycling traits. We estimated the principal components (PCs) of the two–way standardized matrix of six treatments (as combinations of accessions and locations, *n* = 6, *N* = 36), and 12 traits related with productivity (total

TABLE 2 Total N pool for three *Silphium* accessions: HYI, LYI and W for the pre-anthesis and maturity growth stages.

| Growth stages | Total N pool (g N plant^{-1}) | | | $F_{Access.}$ | SED | LSD |
	HYI	LYI	Wild			
Pre-anthesis						
Kansas	1.27 (3.56)	0.47 (1.60)	0.42 (1.52)	13.5***	0.12	0.23
Patagonia	1.90 (6.71)	1.19 (3.30)	1.11 (3.02)			
$F_{Location}$	133.3***					
Maturity						
Kansas	1.22 (3.4)	0.58 (1.8)	0.78 (2.2)	6.4***	0.15	0.30
Patagonia	2.23 (9.3)	0.83 (2.3)	1.43 (4.2)			
$F_{Location}$	7.9***					

Note: For accessions comparison: $n = 12$, because we compare both locations together (no interaction accession × location was found). Standard error of the difference (SED) and least significant difference (LSD) for $p = .05$ and 30 error degrees of freedom are shown. For transformed traits, values are transformed means, and values in parenthesis untransformed means.
HYI, high yield improved; LYI, low yield improved; W, wild.

TABLE 3 Pattern on N allocation as a proportion of the total N pool for pre-anthesis and maturity stages in wild and improved (high-yield [HYI] and low-yield improved [LYI]) accessions of *Silphium* growing in two locations (Kansas, USA and Patagonia, Argentina).

| Locations Accessions | Kansas | | | Patagonia | | | SED | LSD | F_{Access} | $F_{interaction}$ |
	HYI	LYI	Wild	HYI	LYI	Wild				
Pre-anthesis										
CrownNR	0.34 (0.13)	0.41 (0.12)	0.57 (0.30)	0.29 (0.08)	0.24 (0.06)	0.40 (0.16)	0.05	0.02	6.3***	1.79ns
LeafNR	0.77 (0.59)	0.75 (0.57)	0.63 (0.40)	0.92 (0.80)	0.91 (0.84)	0.83 (0.68)	0.06	0.08	9.4***	0.09ns
StalkNR	0.28	0.31	0.30	0.12	0.1	0.16	0.05	0.09	0.4ns	0.05ns
Maturity										
CrownNR	0.38 (0.15)	0.36 (0.13)	0.47 (0.22)	0.42 (0.18)	0.43 (0.19)	0.48 (0.24)	0.09	0.03	17.6**	0.23ns
LeafNR	0.14 (0.02)	0.14 (0.02)	0.14 (0.02)	0.1 (0.01)	0.1 (0.01)	0.1 (0.01)	0.01	0.02	1.3ns	1.27ns
StalkNR	0.23	0.33	0.36	0.30	0.36	0.38	0.10	0.04	6.2***	1.29ns
SuppNR	0.20	0.20	0.19	0.18	0.12	0.16	0.26	0.05	1.8ns	1.91ns
SeedNR	0.40	0.32	0.21	0.33	0.32	0.21	0.13	0.05	17.3***	1.23ns

Note: Leaf at pre-anthesis was green while at maturity all leaves were senescent. For transformed traits, values are transformed means and values in parenthesis untransformed means. Standard error of the difference (SED) and least significant difference (LSD) for p 0.05 and 30 error degrees of freedom are shown for both localities together, $n = 12$. We show F test for accessions (F_{Access}) main effect, and interaction between accessions and locations ($F_{interaction}$).
Abbreviations: CrownNR, crown nitrogen ratio; LeafNR, leaf nitrogen ratio; ns, non-significant; StalkNR, stalk nitrogen ratio; SuppNR, reproductive support structures nitrogen ratio; SeedNR, seed nitrogen ratio.
*$p < .05$.**$p < .01$.***$p < .001$.

biomass and seed yield), N allocation (total N pool, LeafNR, and Pool N Crown), and N recycling (N_{seed} loss, N_{veg} loss, Proficiency, NRE, MRT, NP and NUE). From this analysis, a bi-plot of the first two PCs of treatments and traits was constructed.

Path analysis is a form of multiple regression that allows consideration of complex causal structures with more than one dependent variable on another of a priori-defined direct and indirect causal relationships. A path analysis was performed to evaluate the functional relationships between N allocation differences in pre-anthesis (LeafNR and PoolNCrown), traits related with the recycling strategies at maturity (NRE, N_{seed} loss, N_{veg} loss,

PoolNCrown) and differences in NUE and their components (MRT and NP) between improved accessions (HYI, Figure 2a; LYI, Figure 2b) in relation to Wild for both locations together (N = 24 for each path analysis, 12 plants per accession). The full path model was constructed using as a basis the functional relationships between traits proposed by Silla and Escudero (2004), Yuan et al. (2008), and Masclaux et al. (2001). This Path model was tested using the maximum likelihood Chi-squared statistic with the program package AMOS (Arbuckle & Wothke, 1999). Data that contradict the predicted patterns of covariance, and therefore the hypothesized causal structure of the data, produces a significant

TABLE 4 Traits related with N recycling and N use efficiency for three *Silphium* accessions: High-yield improved (HYI), low-yield improved (LYI) and W-Wild growing in two locations (Kansas, USA and Patagonia, Argentina).

Locations	KANSAS			PATAGONIA			SED	LSD	F_{Access}	$F_{interaction}$
Accessions	HYI	LYI	Wild	HYI	LYI	Wild				
N conservation traits										
[N] senescent (g)	4.36	7.89	10.73	6.2	8.30	8.39	0.67	1.39	20.6***	14.93ns
NRE (%)	63	32.5	26.9	64.4	47.5	37.8	5.54	11.4	17.6***	0.85ns
N_{veg} loss (mg N pl^{-1})	39.2	62.8	45.6	37.6	51.6	31.4	0.01	0.02	15.2**	1.09ns
N_{seed} loss (mg N pl^{-1})	2.5 (12.2)	1.92 (6.8)	1.59 (4.9)	3.02 (20.5)	2.21 (9.1)	2.07 (7.9)	0.19	0.38	4.5**	1.98ns
MRT (weeks)	4.08 (59.2)	4.20 (66.9)	4.68 (108.3)	4.09 (59.9)	3.91 (50.2)	4.69 (109.1)	0.08	0.16	5.1**	0.43ns
PoolNCrown$_{pre-anthesis}$ (mg N pl^{-1})	1.81 (6.08)	1.88 (6.54)	2.12 (8.3)	2.23 (9.3)	2.25 (9.5)	2.68 (14.6)	0.20	0.41	11.6**	1.67ns
PoolNCrown$_{maturity}$ (mg N pl^{-1})	16.9	48.4	57.1	43.1	124.3	129.4	0.34	0.67	12.11*	0.34ns
N use efficiency traits										
NP (g mg^{-1} weeks^{-1})	14.6	9.21	14.1	11.7	9.4	12.3	1.27	2.52	8.6***	3.14ns
NUE (g biomass g^{-1} N)	−0.54 (57.8)	−1.05 (34.8)	0.42 (152.2)	−0.62 (54.2)	−0.53 (59.4)	0.19 (120.7)	0.17	0.36	16.3***	1.32ns
NUtE (g seeds g^{-1} N)	−0.62 (54.2)	−0.48 (62.1)	−0.54 (57.8)	−0.16 (66.8)	−0.29 (75.1)	−0.34 (70.7)	0.18	0.38	1.79ns	0.36ns

Note: For transformed traits, values are transformed means and values in parenthesis untransformed means. Standard error of the difference (SED) and least significant difference (LSD) for *p* .05 and 30 error degrees of freedom are shown for both localities together, *n* = 12. We show *F* test for accessions (F_{Access}) main effect, and interaction between accessions and locations ($F_{interaction}$).

Abbreviations: MRT, mean residence time; NP, nitrogen productivity; NRE, nitrogen resorption efficiency; ns, non-significant. [N]senescent = Proficiency; N_{veg} loss, N lost from the plant due to leaf senescence; N_{seed} loss, N lost from the plant due to harvest; PoolNCrown = the N content stored in the crown at pre-anthesis and maturity; NUE, N use efficiency; NUtE, N utilization efficiency.

*p < .05.**p < .01.***p < .001.

Chi-squared value, indicating that the model must be rejected. A well-fitting model produces a non-significant Chi-squared value. We calculate the descriptive fit indices, CFI and NFI. Values close to 1 are generally considered to indicate a good fit.

3 | RESULTS

3.1 | N allocation patterns

HYI accession had a larger total N pool than that of LYI and Wild accessions in both phenological stages (pre-anthesis and maturity) and locations (non-significant interaction Accession X Location, $F = 3.42$ns and $F = 3.75$ns for pre-anthesis and maturity respectively; Table 2).

At pre-anthesis accessions differed in the proportional allocation of N to vegetative organs: both improved accessions (HYI and LYI) allocated more N to leaves (LeafNR) and less to crown (CrownNR) than the Wild accession. Proportional N allocation to stalks did not differ between accessions (Table 3). These patterns were similar in both locations (non-significant interaction Accession X Location see Table 3).

At maturity, a trade-off in N allocation was found between crown and seeds. HYI and LYI accessions had a lower CrownNR but a higher SeedNR than that of the Wild accession (in both locations; non-significant interaction Accession X Location, see Table 3). Proportional

N allocation to senescent leaves and reproductive support structures did not differ between accessions (Table 3).

When analysed by location, it was found that accessions growing in Kansas had a lower Total N pool than those in Patagonia (Table 2). The proportional N allocation pattern did not differ between locations ($F = 1.37$ns for CrownNR; $F = 0.50$ns for StalkNR; $F = 1.19$ns for LeafNR; $F = 4.69$ns for SuppNR, $F = 0.72$ns for SeedNR).

3.2 | N recycling and N use efficiency traits

HYI resorbed 64% of the N present in the leaves before senescence, and were more efficient in resorption than LYI and Wild (NRE was 36% in average for all two accessions in both locations; Table 4). Proficiency was higher in HYI in relation to the others ([N] in senescent leaves was lower in HYI; Table 4). However, the N lost through leaf senescence (N_{veg} loss) did not differ between HYI and Wild accessions. LYI accessions had the highest N_{veg} loss while HYI had the highest N_{seed} loss in both locations (Table 4). N stored in the crown at pre-anthesis was lower in both Improved accessions (HYI and LYI; both locations), than in Wild plants. At maturity, LYI did not differ from the Wild accession in the PoolNCrown, and it was higher that than of HYI (Table 4).

The mean residence time of N in the plant was 74% higher in Wild than in improved accessions (HYI and LYI, Table 4).

Nitrogen productivity (NP) was lower in LYI than in HYI and Wild accessions, and the wild accession showed the highest NUE. Nitrogen utilization efficiency (NUtE) did not differ between accessions (Table 4).

3.3 | Functional relationships

We compared differences in functional relationships between traits related to N allocation, recycling and N-use-efficiency in high yield (Figure 1a; $X^2 = 22.2$, $p = .11$, df $= 15$) and low yield (Figure 1b; $X^2 = 21.7$, $p = .06$, df $= 13$) improved accessions, compared to Wild. In both improved types (HYI and LYI), we found positive relationships between the proportional allocation to leaves at pre-anthesis and nitrogen recycling efficiency (NRE). Also, NRE was negatively related to nitrogen productivity (NP) and N_{veg} loss, indicating that improved accessions with higher LeafNR were more efficient resorbing N during senescence and had lower N_{veg} loss and NP.

The trade-off between Leaf and Crown N allocation resulted in a lower N allocated to the crown at pre-anthesis in both HYI and LYI (Figures 1a, b). In HYI, the lower N investment in the crown at pre-anthesis was the main determinant of the lower N pool in the crown at maturity (Figure 1a). In the LYI accession, the N pool in the crown at maturity was also influenced by N resorption during senescence (positive relation between NRE and PoolNCrown).

The mean residence time of N in the plant (MRT) was determined by the N storage in the crown in both HYI and LYI accessions (positive relationship between PoolNCrown maturity and MRT). While in LYI, MRT was also modulated by N_{veg} loss (negative relationship, Figure 1b), in HYI the main source of N removal was throughout seed production. Also, NRE was positively related to N_{seed} loss, and N_{seed} loss negatively related to MRT. In both HYI and LYI, NUE was positively linked to both, MRT and NP.

3.4 | Linking N recycling strategies with productivity

In Figure 2, the first PCA axis based on 12 traits related to N economy and productivity accounted for 43% of overall variation and it was linked to differences in N recycling strategy of accessions. With increasing scores on the first PCA axis, variables related to resorption (NRE and Proficiency) and N seed loss increased, while variables representative of N conservation strategies such as MRT, PoolNcrown and NUE, decreased (Table 5). Consistent with this N recycling strategy axis, HYI accessions from both locations were clustered on the higher resorption and N removal through seeds (N_{seed} loss) side of the spectrum while the Wild accession was placed at the opposite extreme with a higher mean residence time, which resulted from a higher storage of N in the crown and higher NUE. LYI accessions from Kansas and Patagonia were spread in-between these two extremes (Figures 2a, b). This axis was not related with any productivity trait (Figure 2, Table 5).

TABLE 5 Pearson correlation coefficients between the 12 traits used in the PCA analysis and the scores in the CP 1 and CP 2.

Traits	Correlation coefficient	
	CP 1	CP2
Total biomass (g)	0.32	−0.89
Seed yield (g)	0.55	−0.79
Total N pool (g N pl^{-1})	0.58	−0.66
N_{seed} loss (g N pl^{-1})	0.86	−0.64
LeafNR	−0.51	−0.76
Pool N crown	−0.74	−0.50
N_{veg} loss	−0.08	0.92
Proficiency	0.68	0.02
NRE	0.90	−0.33
MRT	−0.88	−0.22
NP	−0.52	−0.52
NUE	−0.83	−0.51

Note: Bold numbers indicated the traits that were selected as relevant to define the axis 1 and 2 of the biplot shown in Figure 3a.
Abbreviations: MRT, mean residence time; NP, nitrogen productivity; NRE, nitrogen resorption efficiency; N_{veg} loss, N lost from the plant due to leaf senescence; N_{seed} loss, N lost from the plant due to harvest; NUE, N use efficiency.

The second PCA axis accounted for 37% of overall variation and it was related to productivity and N acquisition (Table 5). LYI accessions were located at the top of the biplot, which characterizes lower productivity (total biomass and seed yield) even in relation to that of the wild accession, lower total N pool and nitrogen productivity and an increased loss of N through senescence (N_{veg} loss; Figures 2a, c). HYI accessions were grouped at the bottom of the biplot with higher productivity, Total N Pool, LeafNR, N_{seed} loss, and lower N_{veg} loss; Wild accession occupied intermediate position between these two extremes.

4 | DISCUSSION

4.1 | Domestication effects on N budget

De novo domestication of new perennial grain crops has been suggested as a technological innovation to enhance ecosystem services at the same time that provide food, fibre, feed and/or fuels (Duchene et al., 2020). This claim is supported by at least three characteristics of perennial species related to their conservative resource-use-strategy. First, at the plant level, perennial species are more efficient than annuals in the use of resources such as N (Dawson et al., 2008; Gonzalez-Paleo et al., 2019) resulting in lower dependence on N fertilizer; second, they have a seasonal N recycling strategy that provides yield stability across years (Pastor-Pastor et al., 2018; Rajnoch et al., 2020; Schwartz & Amasino, 2013). Third, at an agroecosystem level, their root system together with their conservative strategy

based on N recycling, prevents runoff and nutrient leaching (Culman et al., 2013), and also, controls soil fertility through a better regulation of N mineralization. Other ecosystem services have also been put forward for perennial species (Asbjornsen et al., 2014).

However, domestication moves inadvertently wild perennial species towards the acquisitive side of the spectrum of resource use strategies (Milla et al., 2014; Vilela & Gonzalez-Paleo, 2015). Changes have been documented at different levels such as leaf anatomy, structure and physiology (Gonzalez-Paleo & Ravetta, 2011; Milla et al., 2014), C economy and storage (Gonzalez-Paleo et al., 2016), root architecture (Pastor-Pastor et al., 2019); chemical composition and plant defence (Turner et al., 2018), litter quality and decomposition (Garcia-Palacios et al., 2013; Gonzalez-Paleo et al., 2022), among others.

Our goal here was to understand if and how domestication is affecting the N economy in *Silphium integrifolium*. We found that domestication changed three phases of the internal recycling of N: (1) N acquisition and allocation; (2) N resorption from senescent leaves to different sinks (seed or storage organs); and (3) N proficiency with the consequences for the N input to the agroecosystem through litter quality (Figure 3).

4.1.1 | N acquisition and allocation

The increase in total N pool per plant and the changes in the proportional allocation of N provoked by high yield selection in perennial *Silphium*, resemble similar changes in C economy, previously reported by us, that is, an increase in total biomass and aboveground biomass at the expense of belowground allocation (Vilela et al., 2018). The increase in total N pool has been reported in several annual crops (*Zea*, Emmet et al., 2018; *Triticum*, Nehe et al., 2020) as well as in new perennial crops (*Physaria*, Pastor-Pastor et al., 2018), and it has been

linked to a better N uptake capacity. Enhanced resource acquisition capacity, such as nutrients, water and CO_2, is a requirement to support the increase in plant size frequently reported during the domestication process (Milla et al., 2014; Milla & Matesanz, 2017). In *Silphium*, HYI accessions produced more than twice the biomass and acquired twofold more N compared to LYI and Wild accessions, which did not differ in biomass production. This indicates that a limitation in the capacity to acquire N could be responsible in part for the smaller size and lower seed yield in LYI. Contrarily to the goal of reducing N fertilization through the development of more N use efficient perennial crops, our data suggest the need to increase N input to sustain productivity. The nitrogen acquisition capacity required to increase plant size can be enhanced by either increasing the rate of mineral nutrient uptake by the roots, or by the construction of a root system that maximizes the nutrient uptake capacity (de la Riva et al., 2021; Pastor-Pastor et al., 2019). No previous comparative studies have investigated the effects of domestication on nutrient uptake or root architecture in *Silphium*, but in another perennial crop, the increase in plant size, seed yield and N pool in domesticated genotypes, was accompanied by changes in root structure, that is, higher specific root length and thinner roots (Pastor-Pastor et al., 2019). Also, the comparison between locations indicates that plants of all accessions growing in Patagonia had four times more total N pool and threefold more biomass than those growing in Kansas. If N uptake is limiting biomass production and seed yield, differences between locations could be related to the depth of the water table found in Patagonia, which could improve the plant's access to soil N (Vilela et al., 2018). Another indication of this enhanced N uptake in Patagonia is found in the fact that while the total N pool of the plants increases in Patagonia during the reproductive stage, it does not in Kansas, where the soil is drying by the time of reproduction. This access to soil N helps extend the duration of green leaves (prevent early senescence) and should also allow for the higher seed yield found in Patagonia. Nitrogen fertilizers
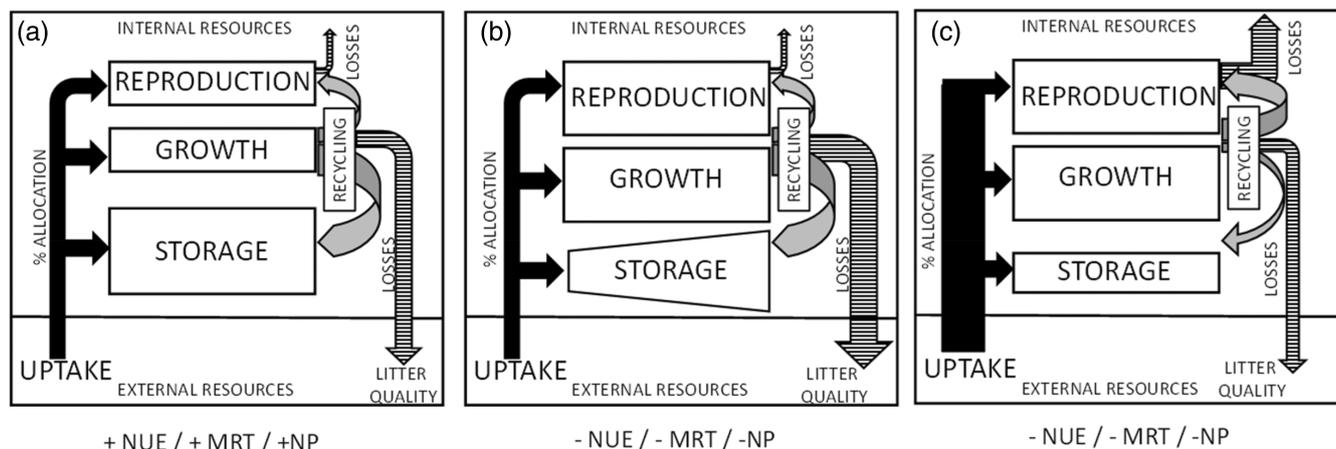
FIGURE 3 Schematic representation of N acquisition and internal cycling in three *Silphium*'s accessions: (a) Wild, (b) LYI, low yield improved and (c) HYI, high yield improved. N uptake is estimated by the N used to construct the total internal pools and their proportional allocation (storage-crown, growth-green leaves and stalks, and reproduction-support structures and seeds) are shown as black arrows. Internal recycling through N resorption form senescent leaves to seeds or storage organs are shown as grey arrows. N losses from the plant through seed or senescent leaves (litter) are shown as striped arrows. The width of the arrows reflects the magnitude of the value of each trait.

experiments in Kansas using improved accessions resulted in an increase in biomass production (mainly during the vegetative stage), and an increase in seed and oil yield. Similar doses to that used for *S. perfoliatum* and sunflower were proposed to optimum biomass production in this environment (120–140 kgN Ha^{-1}; Schiffner et al., 2020). However, these experiments did not evaluate how N addition affects the N internal recycling (resorption, proficiency and storage).

Changes provoked by domestication in the proportional N allocation pattern were similar in both improved accessions (HYI and LYI) and locations, that is, higher allocation to leaves and lower to crown at pre-anthesis and higher allocation to seeds and lower to the crown at maturity. The increase in N allocation to leaves at pre-anthesis in improved accessions, is consistent with the reported shift towards a more acquisitive resource use strategy at the leaf level and fast growth occurred during the domestication of *Silphium*: leaves became thinner, with lower defences and higher photosynthetic rate (Gonzalez-Paleo et al., 2022; Ravetta et al., non-published). On the other side, the wild-leaf type was thicker, with lower photosynthetic rate, lower N concentration and higher investment in chemical defences such as resins (Turner et al., 2018), a typical conservative strategy (sensu Wright et al., 2004) related to slow growth (Reich, 2014) and high- stress resistance (Vilela & Gonzalez-Paleo, 2015). At the plant level, this leaves versus crown trade-off in N allocation can be related to the Growth-Storage trade-off.

The lower N allocation to the crown in improved accessions was maintained through the cycle, and most of this re-allocated N was stored in the seeds, showing a reproduction-storage trade-off (sensu Pastor-Pastor et al., 2018). HYI and LYI allocated 50% more N to seeds in average, reducing a 31% the N allocated to the crown in relation to the Wild. This change in the hierarchy of sinks for N allocation could compromise regrowth in following seasons, and affect plant longevity (Crews et al., 2016; Volenec, 1996) as well as yield-stability across years (Pastor-Pastor et al., 2018). Regrowth and reproduction can use N that is derived from several external (direct root uptake from the soil) or internal sources. Internal resources are originated from storage through the processes of remobilization (crown as an N source) and recycling (crown as an N sink; Millard & Grelet, 2010). A seasonal remobilization of N occurs via translocation from storage pools to other tissues for growth, reproduction and other metabolic functions. We are currently evaluating the importance of C and N remobilized from the crown in relation to current photosynthesis and soil N uptake for growth and reproduction stability with age in *Silphium integrifolium* (Gonzalez-Paleo et al., in preparation). The balance between external and internal N sources determines the nitrogen use efficiency and the fitness of perennial plants in N-poor environments, described below.

4.1.2 | Internal N recycling

Previous research has concluded that the genetic control of N resorption is linked to the regulation of leaf senescence (Masclaux

et al., 2001). Nutrient resorption is a process through which plants reallocate nutrients from senescing structures to storage and/or developing tissues, which contributes to a reduction in their dependence on nutrient uptake (Aerts & Chapin, 2000). Nutrient resorption can assist plants to adapt to environmental variabilities (Prieto & Querejeta, 2020), which can further affect plant growth and reproduction, litter decomposition and ecosystem nutrient cycling through altering nutrient storage and litter quality (Xu et al., 2020).

In our experiments, selection for high yield, enhanced N resorption efficiency only in the HYI accessions, which was significantly higher (60%) than that of Wild (32%) and LYI (40%) accessions. Differences in N resorption between accessions might be dependent on the source: sink ratio which, in our experiment, was strongly decreased by selection only in HYI. Compared to Wild, HYI showed a 65% increment in seed yield and a concomitant 250% increase in the total N allocated to seed (N$_{seed}$ loss) in average for both locations. These results might indicate that nutrient resorption has been positively selected towards a higher efficiency. This has also happened in other improved crops such as *Triticum aestivum*, *Hordeum vulgare*, and in some cultivated trees (Maillard et al., 2015).

During the reproductive phase, seeds are major N sinks in annual plants, while in perennials, roots, stems and crown are also strong sinks for N (Millard & Grelet, 2010). *Silphium* accessions differed in the sink where N from senescent leaves was remobilized (Figures 1a, b). HYI behaved as a typical annual species and improved crops, resorbed N mainly to the seed. The increased N allocation to seed could be an indirect change provoked by selection for seedling vigour and survival in the domesticated accessions of *Silphium* (Vilela et al., 2018). On the other side, in LYI accessions resorbed N was allocated to the crown, restoring the Pool of N at maturity to similar values than that of the Wild accession (Table 4).

When we evaluate the N source: sink relationships in our accessions, the source capacity of the green leaves of improved accessions was higher than that of wild (LeafNratio; Table 3); in relation to the sinks: high yield selection increased the reproductive sink strength only in HYI (Table 2), and in both improved accessions increased the storage sink strength through changes in crown size (data not shown; Crown biomass: $F = 5.9^{***}$; 11.1 ± 0.7 g for improved vs. 6.7 ± 0.5 g for wild accession). Taken together, the accessions differed in the demand for resorbed N by the different sinks: reproductive sink was stronger in HYI while in LYI storage sink was the most important, although this could not be completely filled by the lower total N pool of this accession.

Both annuals and perennials have the ability to recycle nitrogen for growth throughout the season, but perennials differ by having two alternative sinks for N resorbed at the end of the season: the seeds (acropetal) and the crown/root system (basipetal). Thus, some exogenous and/or endogenous factors promote translocation downward in perennials in the second half of the growing season (Schwartz & Amasino, 2013). The trigger could be the initiation of the seed-filling period, changes in photoperiod, or a stronger growth of the crown and roots, which creates a higher sink. Determining how the crown

and roots become a sink for nutrients is imperative to tailor N recycling for specific crops and environments.

The significance of having N reserves lies in the conservation of internal N that allows plants: (1) to reutilize for new growth at resprouting (Pastor-Pastor et al., 2018), when external resources are limiting or N mineralization rate is low (Deng et al., 2018); (2) to accumulate N and avoid its loss from the soil through leaching in periods when availability exceeds demands; (3) to increase the residence time of N, which is particularly important for species growing under nutrient-poor conditions or in low-input agricultural system (without fertilizer uses). Nitrogen use efficiency and mean residence time of N were reduced in both improved accessions (Table 5). In HYI, the lower MRT was consequence of the lower storage of N in the crown and N removal through seed production (Figure 1a), while in LYI was the result of the N lost through leaf senescence (Figure 1b).

4.1.3 | C. Proficiency and N input to the ecosystem

Because of limitations to hydrolyse and resorb some structural proteins present in the senescent leaves, not all of the nitrogen present in green leaves can be kept by the plant during resorption. That 'extra' N (measure with the proficiency index) present in dead leaves, is then recycled through soil decomposers, before it is again available for plant uptake. In *Silphium*, HYI accessions were more proficient (i.e., they had lower N concentration in senescing leaves) than the Wild accession, while LYI accessions were intermediate. These differences in N concentration in senescent leaves should have consequences in the rate of decomposition of this leaf litter and on other soil chemical variables, since around 75% of the variation in litter N mineralization can be explained by variation in litter N concentration (Parton et al., 2007). It has been proposed that net N release starts when the C/N ratio of litter decreases below 40 (corresponding to 1% of N in the litter dry mass; Watson et al., 2002). Above this C/N ratio microbes need to absorb additional N for growth and N is immobilized in their biomass. For *Silphium*, we have reported an increase in C/N ratio and a decrease in N concentration in the litter of HYI accession in relation to the Wild progenitor (Gonzalez-Paleo et al., 2022). Our results now suggest that the lower N concentration in the leaf litter of HYI could decrease the net N mineralization rate and N release to the soil, lowering, in the long term, soil N availability. Hence, N resorption which is highly beneficial for plant growth and yield stability (May et al., 1992), could create a tradeoff between root N uptake and plant growth by means of reducing soil N availability (Aerts, 1996; Aerts & Chapin, 2000).

Although both resorption and mineralization make N available to plants, the two strategies would produce important differences in N cycling at the ecosystem level:

(A) *HYI accessions*: on the one hand, the higher N resorbed, normally considered a conservative trait (Cornwell & Cornelissen, 2013), was not related with a better internal cycling because it was not stored in the crown but removed from the plant and the agroecosystem during the seed harvest. Thus, this resorbed N is not available for future plant growth. On the other hand, the leaf litter type of HYI accessions would allow for slower soil N-cycling rates (lower N mineralization) than the Wild and LYI litter type. In the long run, at some point in the multiannual cycle of production, due to their higher N requirements and higher allocation to leaves to sustain the C gain and productivity (Ravetta et al., unpublished), HYI plants should require higher external N inputs to the soil to sustain their N uptake requirements, than LYI and Wild accessions.

(B) *LYI and Wild accessions*: despite the lower resorption efficiency, N resorbed was allocated to the crown for future remobilization and uses (better internal cycling). The leaf litter type of these accessions with higher N content should sustain faster N-cycles rates (N mineralization) and N release to the soil, enhancing soil fertility. These results do not support the assumption that wild perennial species produce low-quality litter linked to a conservative resource-use strategy. Still, previous findings have shown that Wild accession of *Silphium* which produces leaf litter with higher N content than HYI, but also had a slow decomposition rate as a consequence of the higher resin content (Gonzalez-Paleo et al., 2022) which is being reduced in selected types (Vilela et al., 2018).

The consequences of changes in N economy and secondary metabolites concentration during domestication on N mineralization/immobilization rates and their effects on the plant–litter–soil–plant feedback, need to be evaluated to predict potential effects on N uptake strategies (external versus internal) in perennial crop *Silphium*.

4.2 | Linking internal *n* recycling and productivity

The previously reported increase in seed yield of HYI accessions during domestication, can be related mainly with a change in N uptake capacity (higher total N pool), a higher proportional allocation of N to green leaves during pre-anthesis (LeafNR), a lower N_{veg} loss through leaf senescence, and a higher allocation of resorbed N to the seeds (N_{rep} loss), represented by the axis 2 In the PCA (Figure 2). LYI accessions had a lower productivity which was concomitant with their relatively low N uptake and allocation (lower than HYI and similar to that of the Wild accession). These LYI accessions also had a higher loss of N through senescence (the total biomass of senescent leaves was similar to that of HYI but its proficiency was lower). Overall, the differences in N recycling strategy were independent of plant productivity: LYI accessions had an intermediate strategy compared to the Wild (with high MRT prioritizing N recycling to the crown storage, low proficiency and high NUE) and HYI accessions (high NRE, high proficiency and a recycling strategy prioritizing seeds).

5 | CONCLUSION

After 7 years of selection using seed yield-related characters as the criteria, the improved high-yielding accession of the new perennial crop *Silphium integrifolium* showed several physiological changes determining N budget and recycling at the plant level: (1) High

productivity was achieved by a higher N uptake capacity, and higher N allocation to photosynthetic tissue at pre-anthesis. (2) Lower allocation and recycling of N to the storage pool in the crown, which could compromise the capacity to sustain biomass and seed production with age (yield stability), or would require external (fertilizer) N inputs. (3) Higher resorption efficiency (NRE) and N resorbed allocated hierarchically to seeds, which results in high seed yield and high seed quality (protein content). (4) Higher resorption proficiency which means that senescent leaf biomass has low N content and contributes with lower quality litter to the soil.

If the focus is on the provision of ecosystem services, HYI accessions of *Silphium*, could help achieve a reduction in nitrate leaching by maximizing N uptake, N resorption and N removal through seed (i.e. nitrate catch crop), and also produce crop residues that slow-down biogeochemical cycling and soil nutrient retention. In arid and semi-arid ecosystems slow release of nutrients is a strategy that allows to couple N mineralization and release with plant N uptake.

The lack of achievable high yields by LYI accession, could in part be due to a low N uptake capacity, as total N content was lower in these plants than in HYI. Root traits of these two extremes (HYI and LHI) should be studied, in particular those traits related to N acquisition and photosynthetic performance.

Additional criteria to those directly determining of seed yield could be incorporated in the domestication program of *Silphium* to develop special lines for specific purposes. We propose the following: (1) to select for a low NRE and low proficiency to develop lines that produce high-quality crop residues to increase soil fertility; (2) to select for low N allocation to seed (the alternative sink is the crown) to develop lines with higher recycling capacity that result in lower dependence of fertilizer and high yield stability.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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