Sublethal effects of metal toxicity and the measure of plant fitness in ecotoxicological experiments

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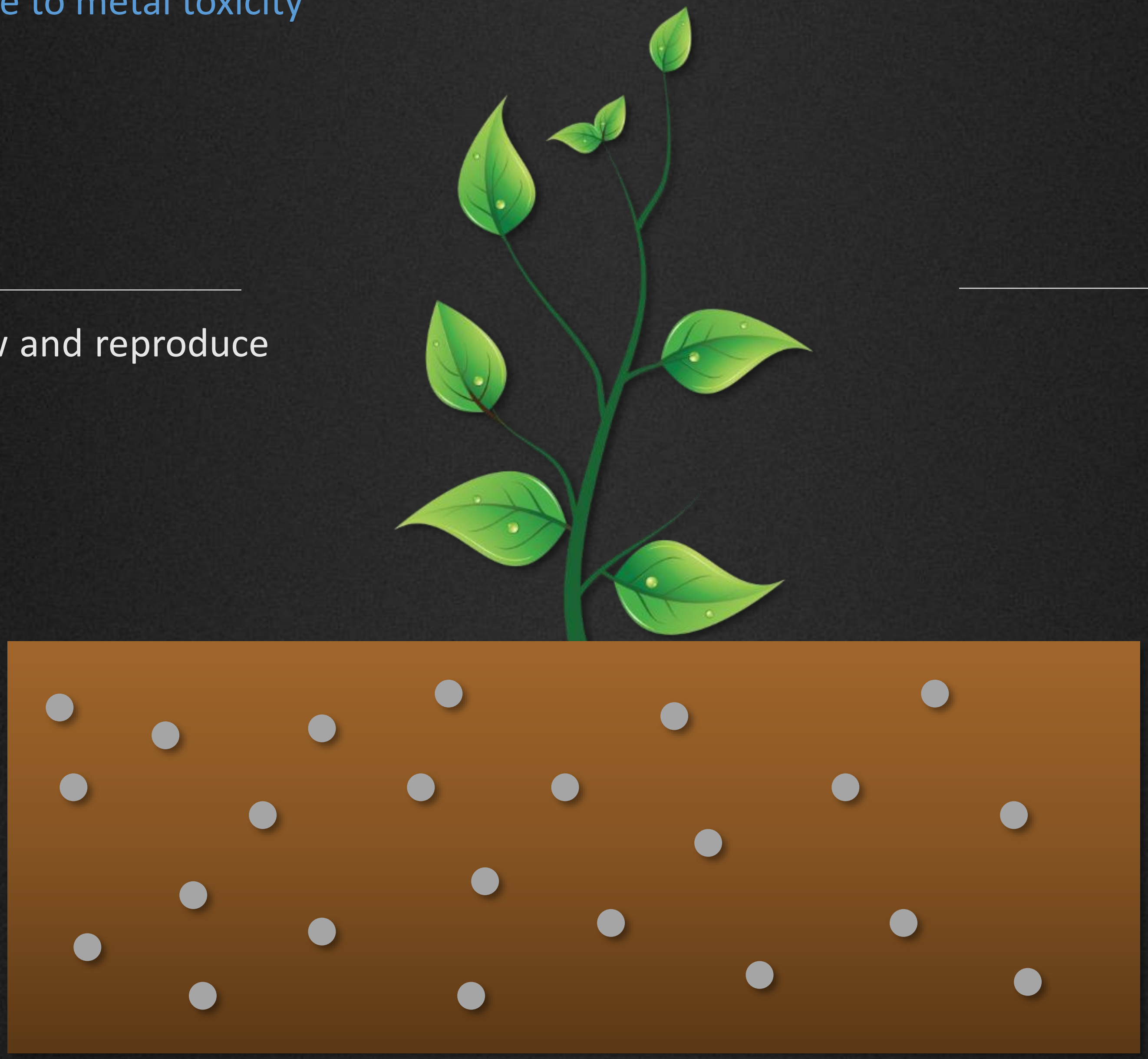
Context : Tolerance to metal toxicity

Metal Tolerance

Ability of a plant to grow and reproduce in metal enriched soil [1]



metallophyte species

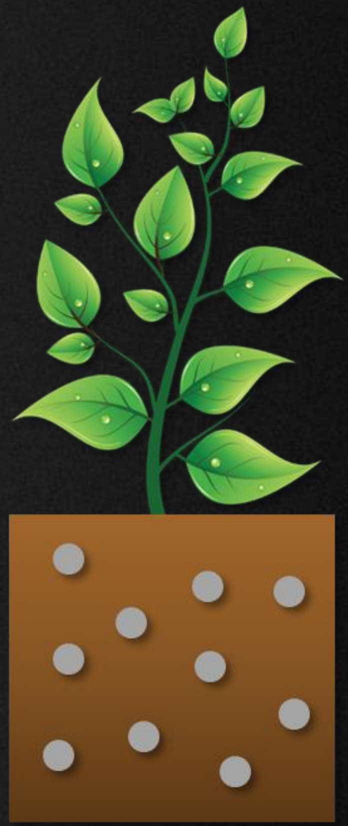
Only on contaminated soil

[1] **Macnair MR**. **1993**. The genetics of metal tolerance in vascular plants. *New Phytologist* **124**: 541–559.

Context : Pseudometallophyte species and local adaptation

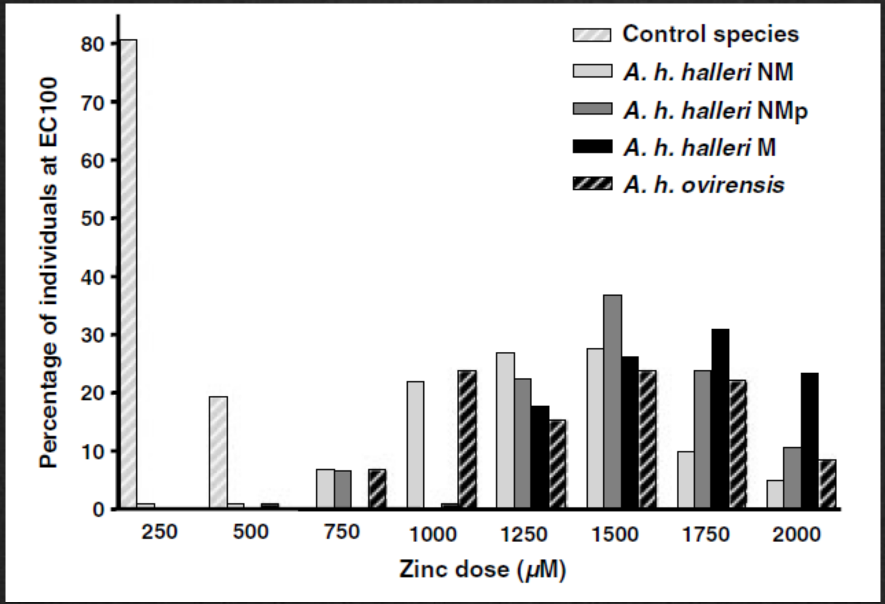
Pseudometallophyte species

Two ecotypes



Non-metallicolous populations in non contaminated soils ≠ **Metallicolous** populations in metal contaminated soils

Constitutive metal tolerance



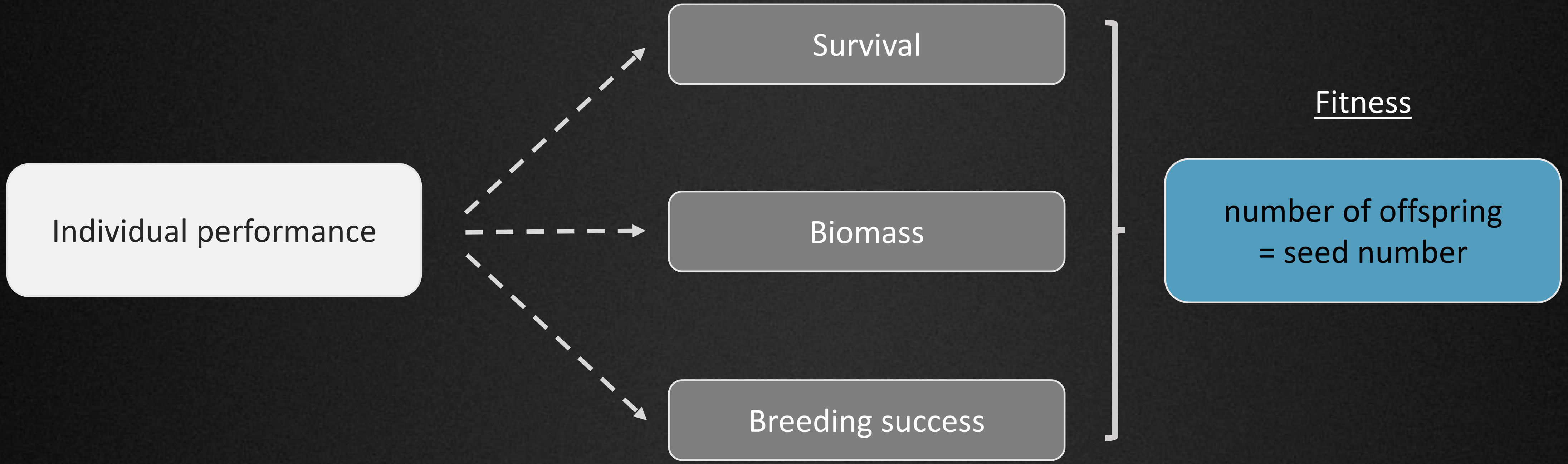
Pauwels et al., 2006, JEB

→ **plant models to study local adaptation to metal toxicity, especially sublethal effects (i.e. fitness)**

Context : How estimating fitness ?

Fitness : Individual capacity to reproduce and propagate his gene in a population [4]

Estimated with 3 components of individual performance [5]



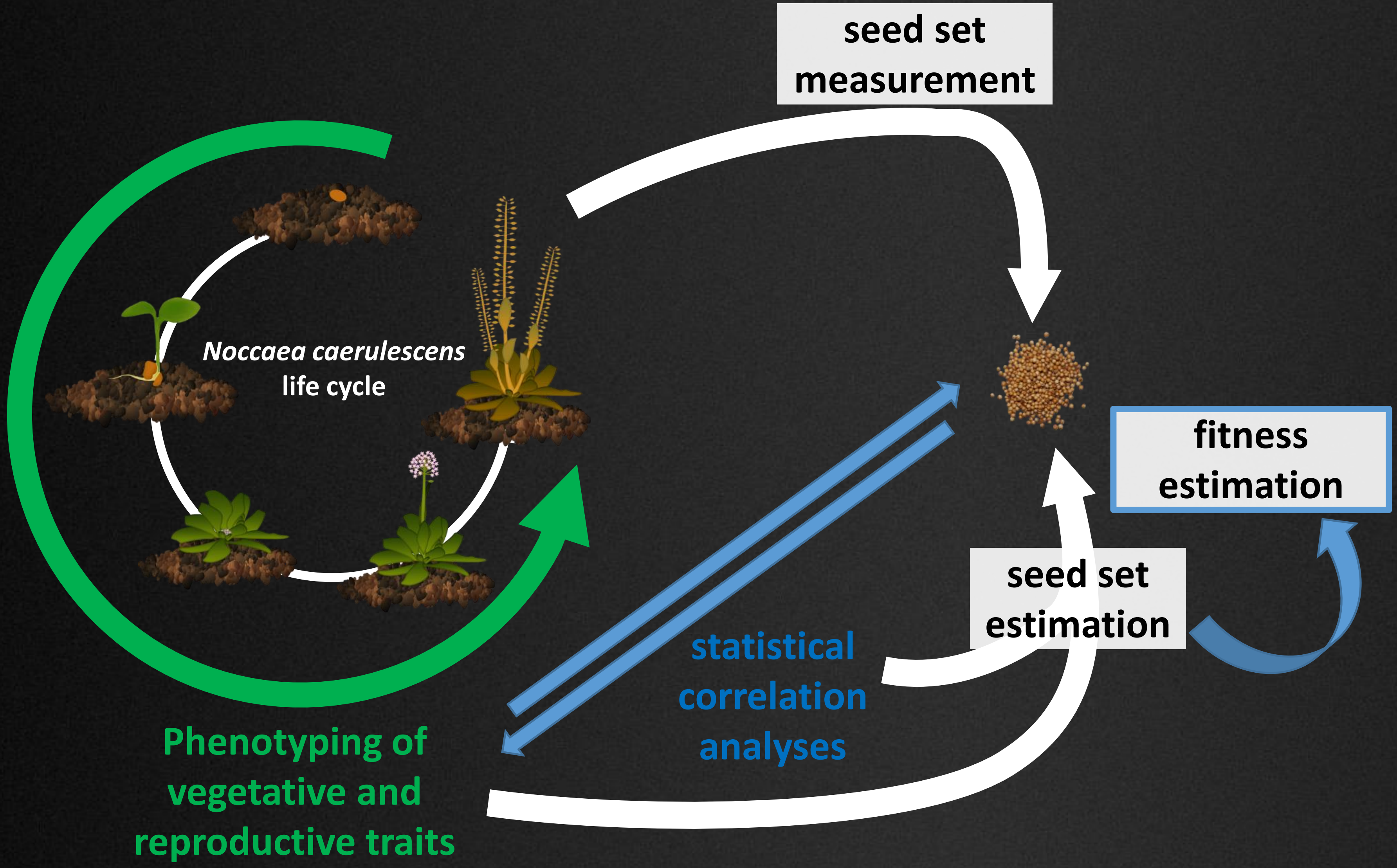
Unable to collect seeds exhaustively → Estimation with allometric experiment

[4] **Westneat DF, Fox CW (Eds.). 2010**. *Evolutionary behavioral ecology*. Oxford ; New York: Oxford University Press.

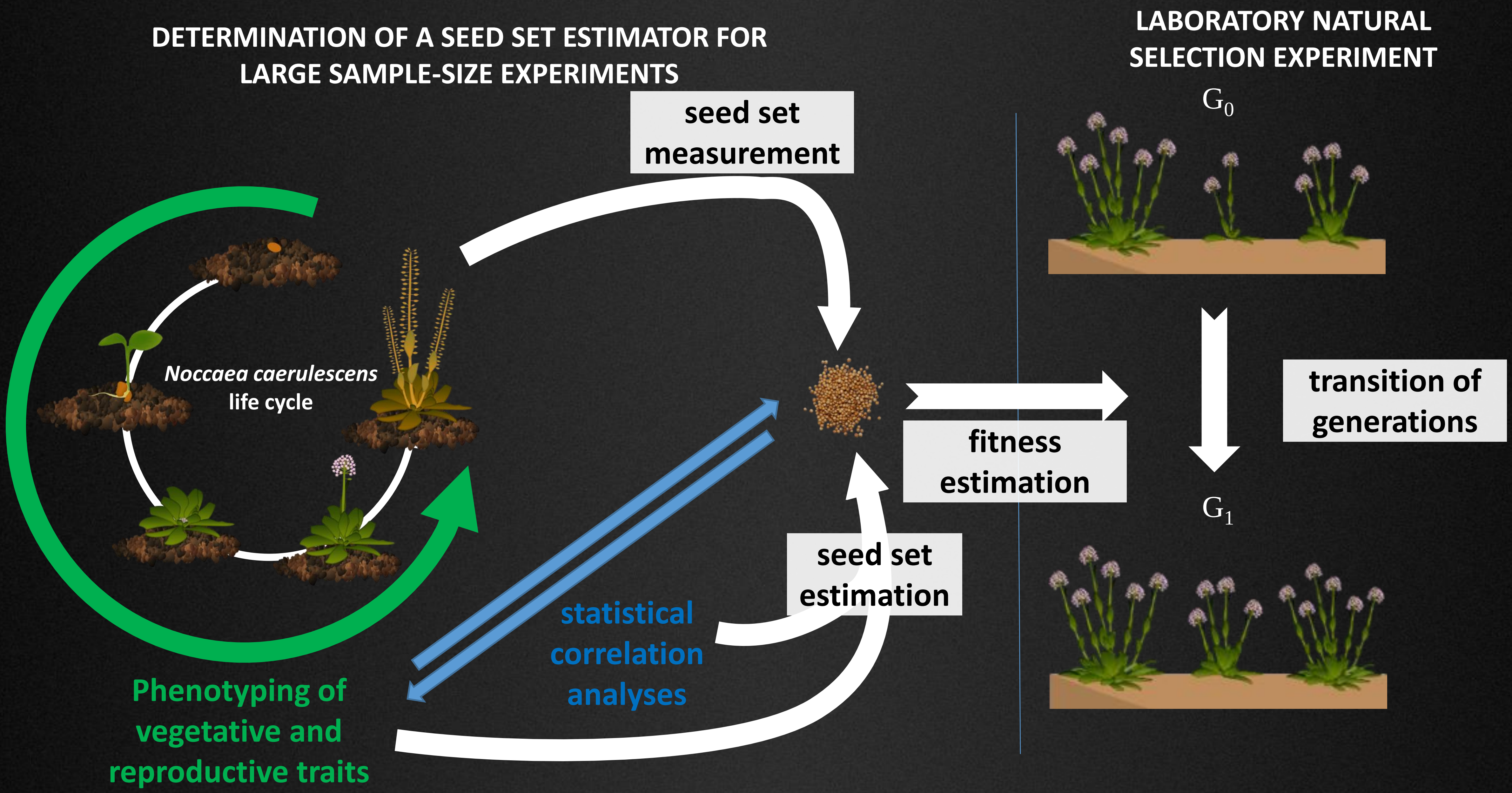
[5] **Violle C, Navas M-L, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E. 2007**. Let the concept of trait be functional! *Oikos* **116**: 882–892.

◆ **Aim** : Estimating fitness in *Noccaea caerulescens* to investigate sublethal effects of metal toxicity

DETERMINATION OF A SEED SET ESTIMATOR FOR LARGE SAMPLE-SIZE EXPERIMENTS



◆ Aim : Estimating fitness in *Noccaea caerulescens* to investigate sublethal effects of metal toxicity



Material and methods

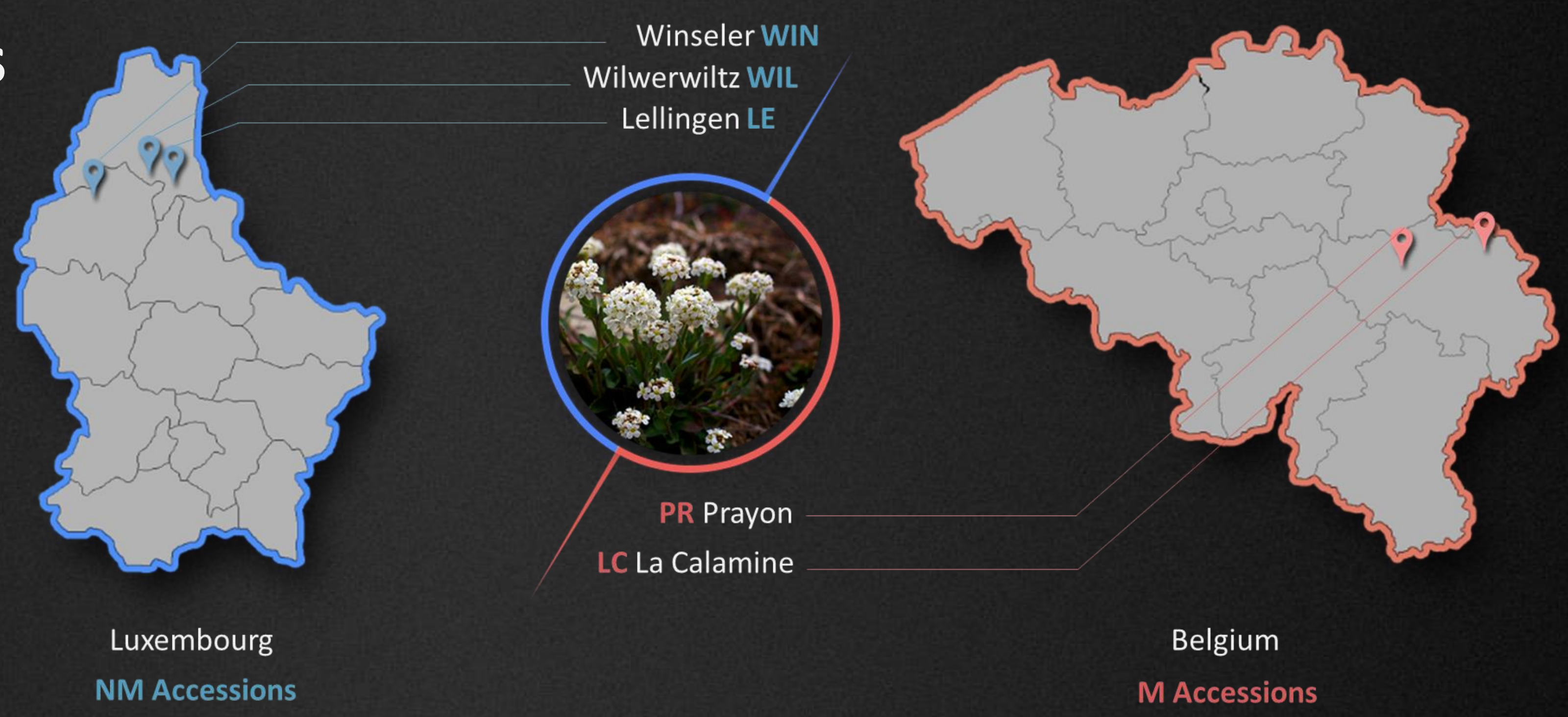


Table S1: Characteristics of the study sites. Metal concentrations are expressed as total concentrations (in mg.kg^{-1}) contained in sampling soils.

	Lellingen[1]	Wilwerwiltz[2]	Winseler[2]	La Calamine[1]	Prayon[3]
Abbreviation	LE	WIL	WIN	LC	PR
Geographic coordinates	49°59′ N. 6°00′ E	49°57′ N. 5°53′ E	49°58′ N. 5°59′ E	50°41'44 N. 05°59'39 E	50°34′52 N. 5°40′02 E
pH	5.7	5.9	5.8	6.8	6.9
Zn	126 ± 4.3	139	164 - 274	101563 ± 14329	75700 ± 13500
Cd	< 1± 0.0	< 2	0.7 - 4.3	217 ± 59	667 ± 85
Pb	48 ± 3.5	54	80 – 136	8998 ± 2524	9620 ± 1460
Ni	48 ± 2.6	42	66 - 157	8998 ± 2524	211 ± 57

[1](Ana G.L. Assunção et al., 2003) [2](Reeves et al., 2001) [3](Roosens et al., 2003)

Material and methods

Lellingen	Wilwerwiltz	Winseler	La Calamine	Prayon
LE.02	WIL.10	WIN.02	LC.05	PR. 02
LE.03.1	WIL.13	WIN.04	LC.06	PR.04
LE.03.2	WIL.15	WIN.05	LC.08	PR.06
LE.07	WIL.18	WIN.06	LC.09	PR.09
LE.11	WIL.19	WIN.07	LC.10	PR.10
LE.12.1	WIL.22	WIN.08	LC.13	PR.13
LE.12.2	WIL.24	WIN.13	LC.15	PR.15
LE.15	WIL.25.1	WIN.14	LC.17	PR.23
LE.16	WIL.25.2	WIN.17	LC.18	PR.28
LE.17	WIL.26	WIN.18	LC.26	PR.30
LE.19	WIL.27.1	WIN.19	LC.27	PR.31
LE.20	WIL.27.2	WIN.21	LC.28	PR.37
LE.24	WIL.30	WIN.22	LC.32	PR.39
LE.27	WIL.33	WIN.24		PR.40
LE.28	WIL.34	WIN.26		
LE.02.1	**WIL.27.1**	**WIN.21.1**	**LC.02**	**PR.21.1**
LE.28	**WIL.34.1**	**WIN.26.1**	**LC.08**	**PR36.1**
			LC.36	**PR.36.2**

List of individuals used for phenotypic survey and involved in controlled crosses. LE=Lellingen, WIL=Wilwerwiltz, WIN=Winseler, LC = La Calamine and PR=Prayon. Individuals in bold characters were used in controlled crosses.

72 wild plants cultivated in plastic tunnel

360 progenies cultivated in greenhouse on zinc polluted and non-polluted soils

List of controlled crosses. M=metallicolous, NM=nonmetallicolous, LE=Lellingen, WIL=Wilwerwiltz, WIN=Winseler, LC = La Calamine and PR=Prayon

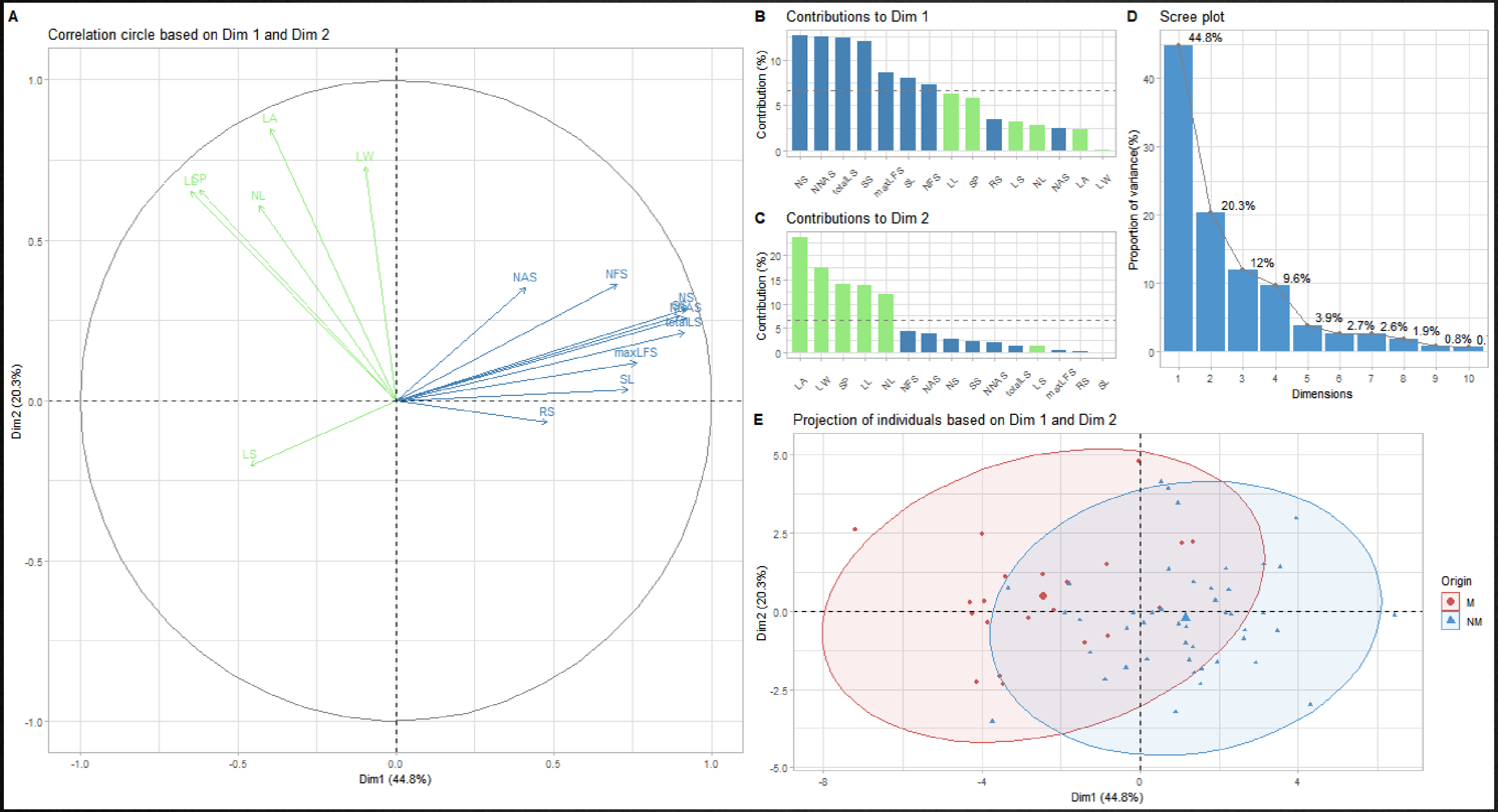
	Cross type	Cross origin		Cross pedigree
Self progenies	M self	PR*PR	PR	PR.36.1*PR.36.1
	M self	PR*PR		PR.36.2*PR.36.2
	M self	PR*PR		PR.21*PR.21
	M self	LC*LC	LC	LC.02*LC.02
	M self	LC*LC		LC.36*LC.36
	M self	LC*LC		LC.08*LC.08
	NM self	LE*LE	LE	LE.28*LE.28
	NM self	LE*LE		LE.02*LE.02
	NM self	WIL*WIL	WIL	WIL.34*WIL.34
	NM self	WIL*WIL		WIL.27*WIL.27
	NM self	WIN*WIN	WIN	WIN.26*WIN.26
	NM self	WIN*WIN		WIN.21*WIN.21
Outbred progenies	M*NM	PR*LE	C1	PR.36.1*LE.28
	NM*M	LE*PR		LE.28*PR.36.1
	M*NM	PR*WIL	C2	PR.36.2*WIL.34
	NM*M	WIL*PR		WIL.34*PR.36.2
	M*NM	PR*WIN	C3	PR.21*WIN.26
	NM*M	WIN*PR		WIN.26*PR.21
	M*NM	LC*WIN	C4	LC.02*WIN.21
	NM*M	WIN*LC		WIN.21*LC.02
	M*NM	LC*LE	C5	LC.36*LE.02
	NM*M	LE*LC		LE.02*LC.36
	M*NM	LC*WIL	C6	LC.08*WIL.27
	NM*M	WIL*LC		WIL.27*LC.08

Material and methods

- **Vegetative traits**
  - ✓ number of leaves (NL)
  - ✓ Rosette diameters -> surface of the plant (SP)
  - ✓ length and width of 3 largest leaves -> leaf shape (LS = LL/LW)
    -> leaf area (LA = $\frac{LL}{2} * \frac{LW}{2} * \pi$)
- **Reproductive traits**
  - ✓ number of flower stems (NFS)
  - ✓ Length of the largest flower stem (maxLFS)
  - ✓ Number of silicles (total (NS), aborted (NNAS), non aborted (NAS))
  - ✓ ratio between NNAS and NAS (RS)
  - ✓ Mean silicle length (estimated from 5 fruit per stem, SL)
  - ✓ Total length of silicles (totalLS = $NNAS * SL$)
  - ✓ Seed set (SS)

Eventually bagging flower stems at early bloom

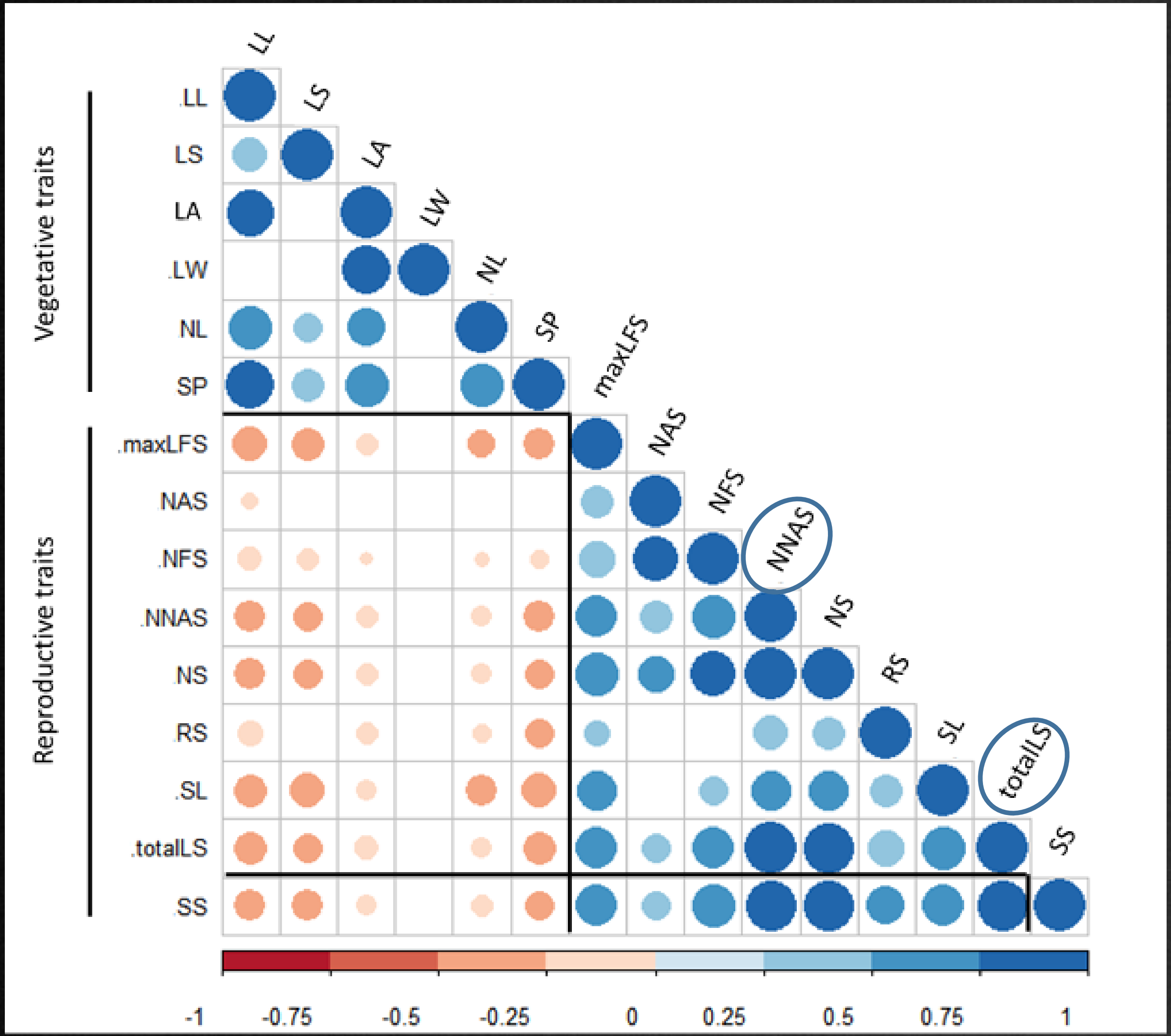
Fitness estimation : Analyses and Results



Reduction of variable number → **Multivariate analysis (PCA)** and **correlation coefficients** between variables

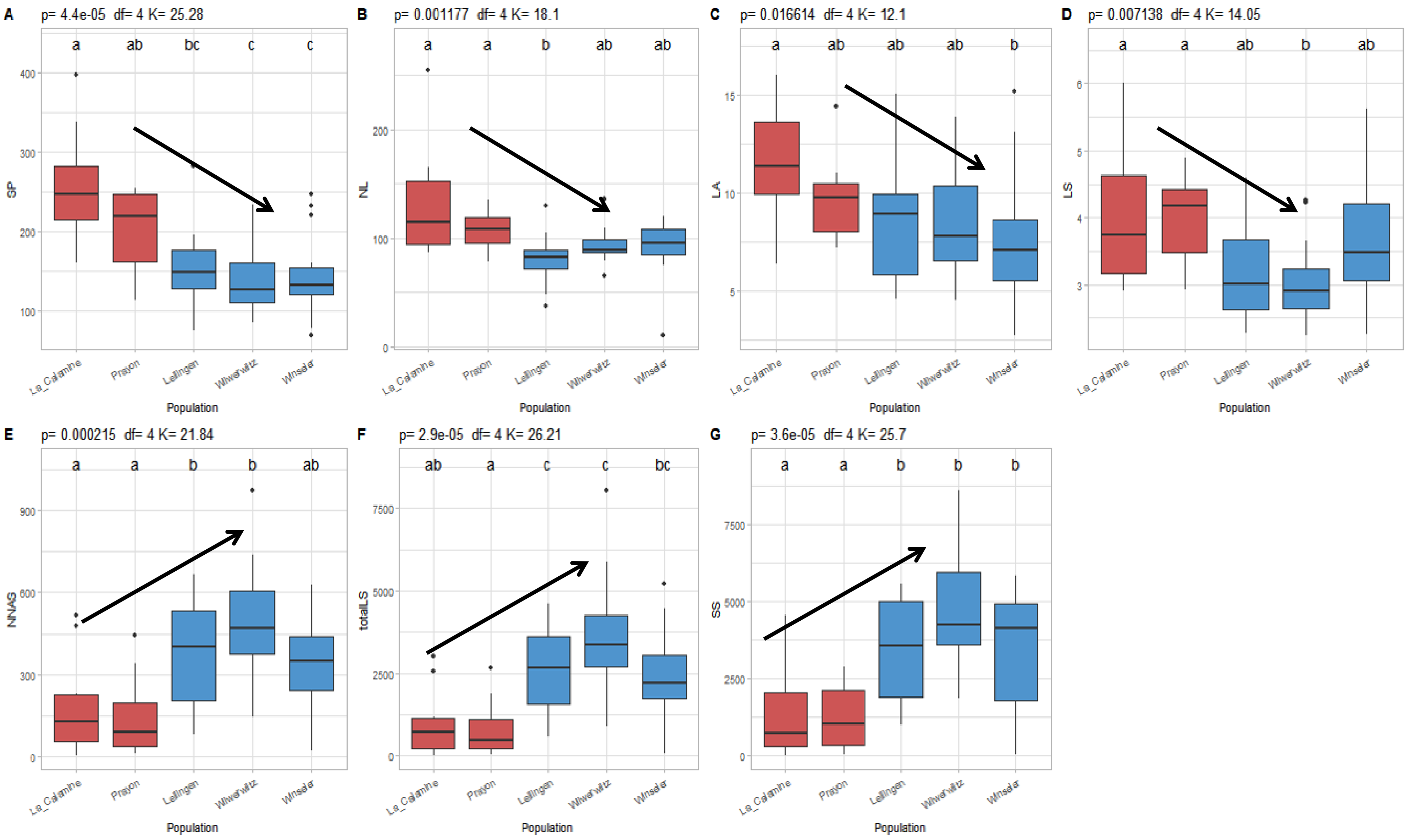
Results of the principal component analysis performed on the 72 wild plants including the correlation circle based on dimension 1 and dimension 2 (A), the variable contributions to dimension 1 (B), the variable contribution to dimension 2 (C), the scree plot of eigenvalues (D) and the projection of individuals based on dimension 1 and dimension 2 according to the edaphic origin of plants (E). M=metallicolous; NM=non-metallicolous. Vegetative traits: SP=surface of the plant, LL=leaf length, LW=leaf width, LS=leaf shape, LA=leaf area, NL=number of leaves. Reproductive traits: SS=seed set, NFS=number of flower stems, maxLFS=length of the longest stem, NS=number of silicles, NAS=number of aborted silicles, NNAS=number of non-aborted silicles, RS=ratio between NNAS and NAS, SL=silicle length, totalLS=total length of silicles. Ellipse surfaces grouped 90% of individuals for each origin. For B and C, the dotted line represents the contribution if all the variables contributed equally.

Fitness estimation : Analyses and Results



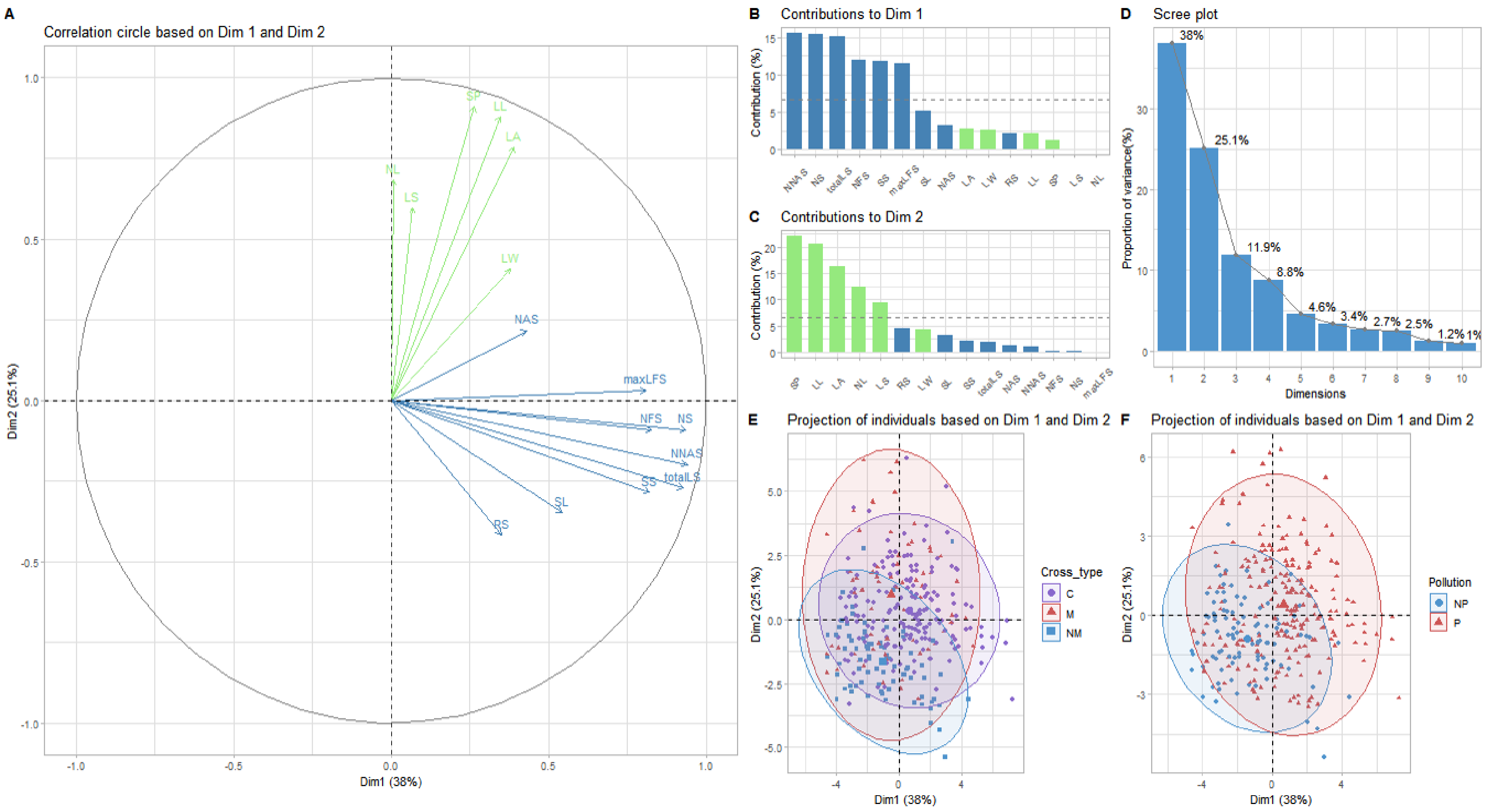
Correlation matrix among all vegetative and reproductive traits in the 72 native plants. The sizes of the circles and the intensities of the colors are proportional to the strength of the correlation. Vegetative traits: SP=surface of the plant, LL=leaf length, LW=leaf width, LS=leaf shape, LA=leaf area, NL=number of leaves. Reproductive traits: SS=seed set, NFS=number of flower stems, maxLFS=length of the longest stem, NS=number of siliques, NAS=number of aborted siliques, NNAS=number of non-aborted siliques, RS=ratio between NNAS and NAS, SL=silique length, totalLS=total length of siliques.

Fitness estimation : Analyses and Results



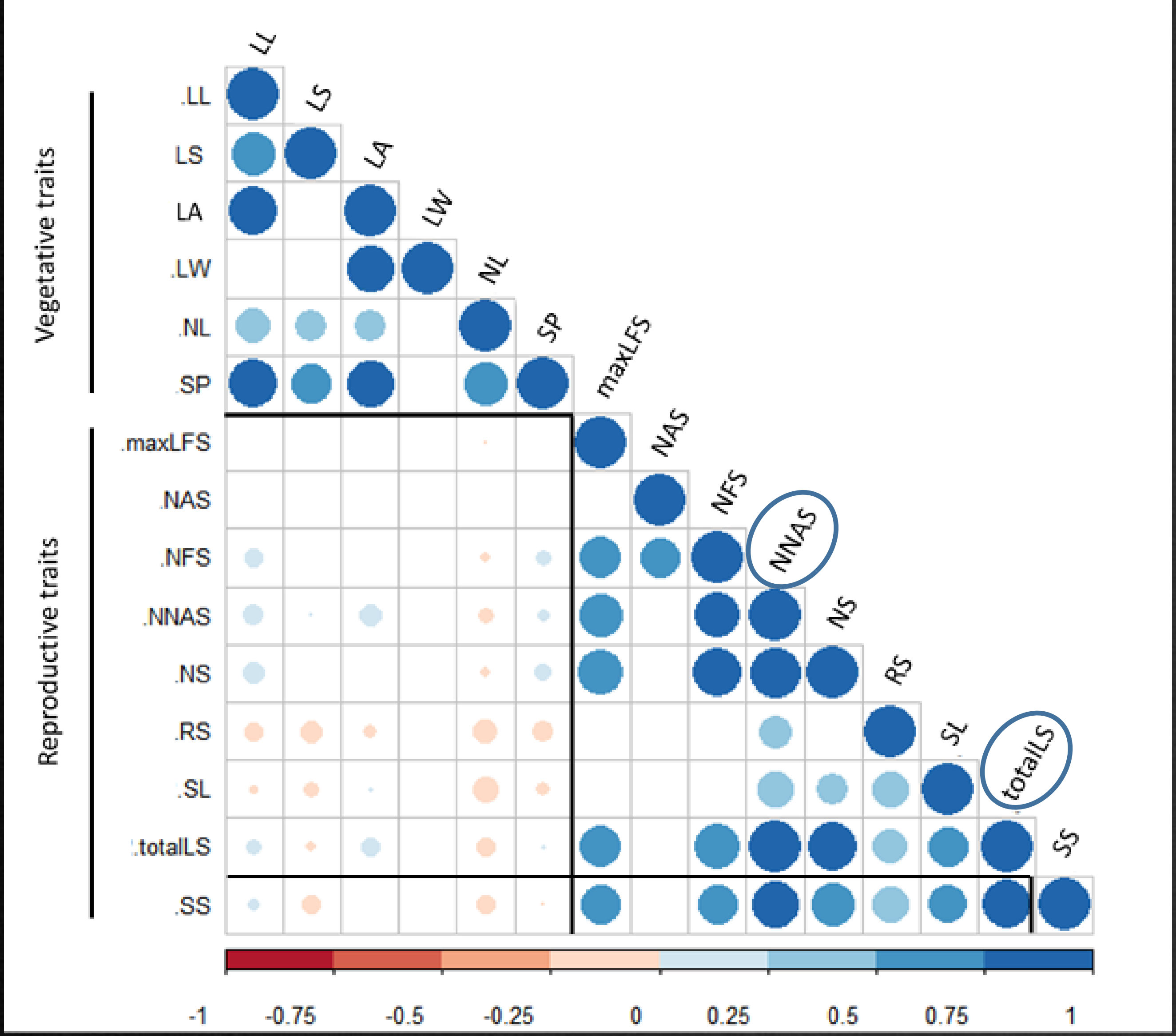
Boxplots and statistical results for some vegetative (A, B, C, D) and reproductive (E, F, G) traits in the 72 wild plants from the two metalliferous sites (red boxes) and the three non-metallicolous sites (blue boxes). Vegetative traits: SP=surface of the plant, LS=leaf shape, LA=leaf area, NL=number of leaves. Reproductive traits: SS=seed set, NNAS=number of non-aborted silicles, totalLS=total length of silicles. The statistical results above each graph correspond to those of the Kruskal-Wallis test. K= Kruskal-Wallis statistics, df= degree of freedom, p= p-value. Letters above each boxplot indicate significant differences at the 5% threshold for the post hoc Conover test.

Fitness estimation : Analyses and Results



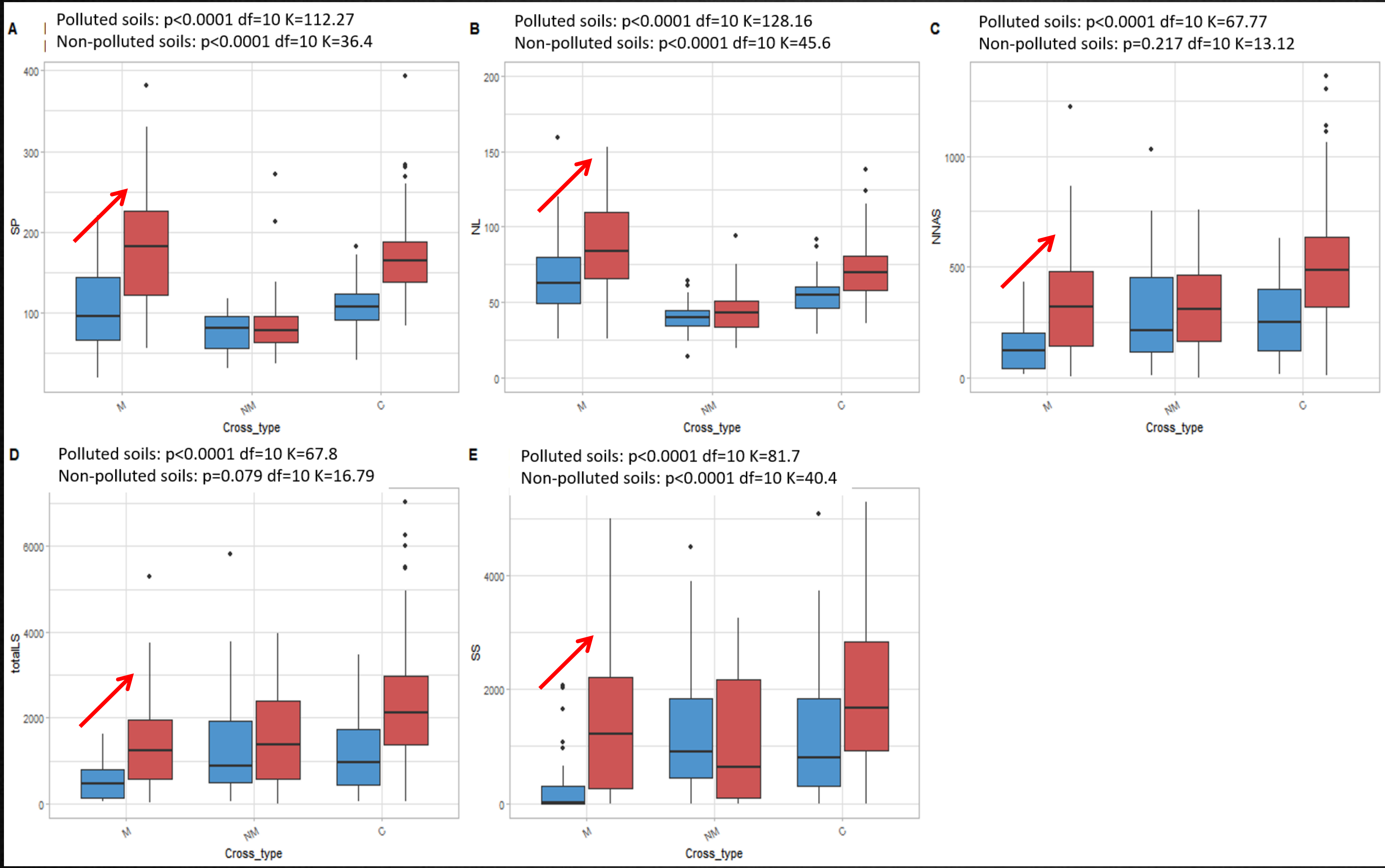
Results of the principal component analysis performed on self- and outbred progenies including the correlation circle based on dimension 1 and dimension 2 (A), the variable contributions to dimension 1 (B), the variable contribution to dimension 2 (C), the scree plot of eigenvalues (D), the projection of individuals based on dimension 1 and dimension 2 according to the cross type (E) and the projection of individuals based on the dimension 1 and dimension 2 according to pollution level (F). M=metallicolous self-crosses; NM=non-metallicolous self-crosses; C=outbred crosses. Vegetative traits: SP=surface of the plant, LL=leaf length, LW=leaf width, LS=leaf shape, LA=leaf area, NL=number of leaves. Reproductive traits: SS=seed set, NFS=number of flower stems, maxLFS=length of the longest stem, NS=number of silicles, NAS=number of aborted silicles, NNAS=number of non-aborted silicles, RS=ratio between NNAS and NAS, SL=silicle length, totalLS=total length of silicles. Ellipse surfaces grouped 90% of individuals for each origin and each pollution level. For B and C, the dotted line represents the contribution if all the variables contributed equally.

Fitness estimation : Analyses and Results



Correlation matrix among all vegetative and reproductive traits in the inbred and outbred progenies. The sizes of the circles and the intensities of the colors are proportional to the strength of the correlation. Vegetative traits: SP=surface of the plant, LL=leaf length, LW=leaf width, LS=leaf shape, LA=leaf area, NL=number of leaves. Reproductive traits: SS=seed set, NFS=number of flower stems, maxLFS=length of the longest stem, NS=number of siliques, NAS=number of aborted siliques, NNAS=number of non-aborted siliques, RS=ratio between NNAS and NAS, SL=silique length, totalLS=total length of siliques.

Fitness estimation : Analyses and Results

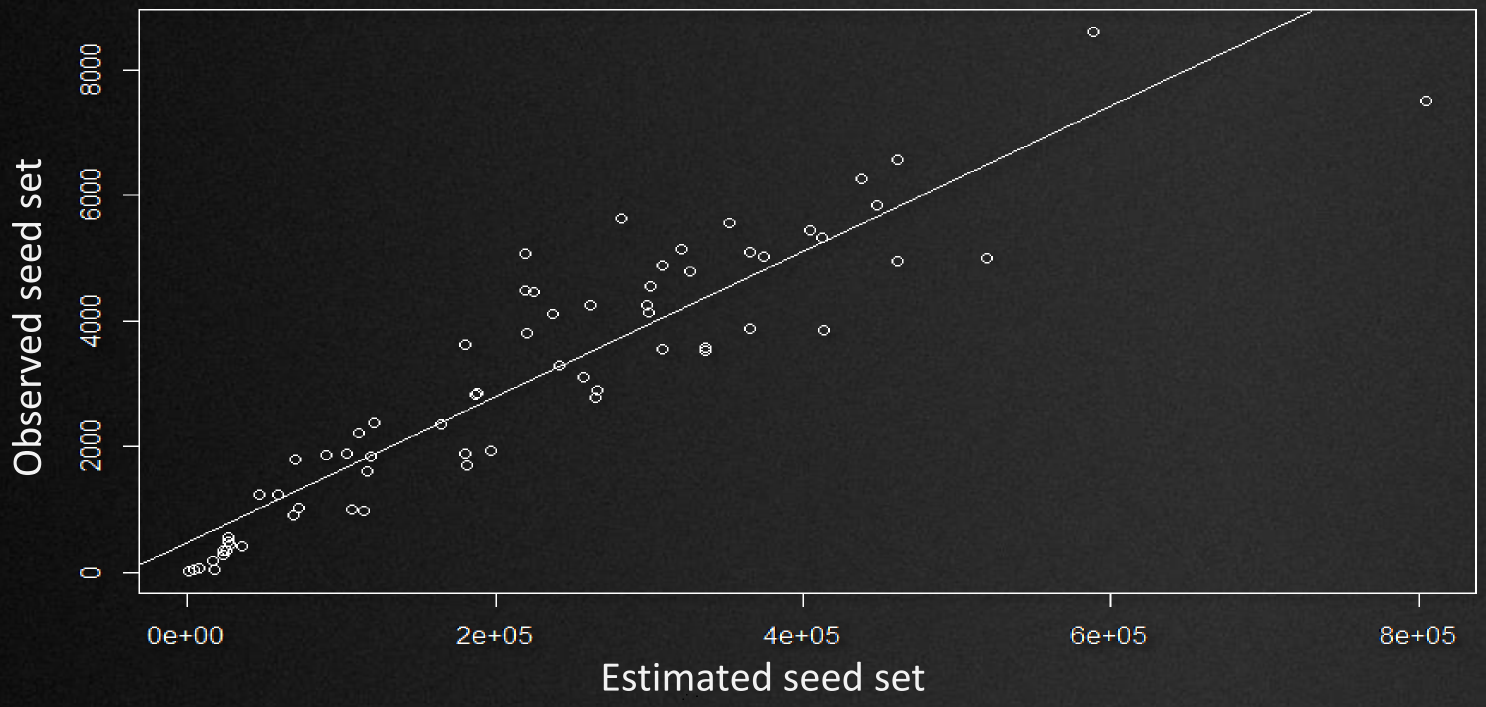


Boxplots and statistical results for some vegetative (A, B) and reproductive (C, D, E) traits in self- and outbred progenies in non-polluted (blue boxes) and polluted (red boxes) soils. Vegetative traits: SP=surface of the plant, NL=number of leaves. Reproductive trait: NNAS=Number of non-aborted silicles, totalLS=total length of silicles, SS=seed set. The statistical results above each graph correspond to those of the Kruskal-Wallis test for the non-polluted soil (blue boxes) and the polluted soil (red boxes). K= Kruskal-Wallis statistics, df= degree of freedom, p= p-value.

Fitness estimation : Analyses and Results

Creating a composite variable that takes into account the number and size of fruits

Estimated seed set = number of non-aborted seeds x total length of silicles



- ✓ Strong correlation (r=0,93 ***) with the observed seed set
- ✓ This variable was also used to estimate the number of seeds in other species [8]

[8] **Brachi B, Aimé C, Glorieux C, Cuguen J, Roux F. 2012**. Adaptive Value of Phenological Traits in Stressful Environments: Predictions Based on Seed Production and Laboratory Natural Selection (V Laudet, Ed.). *PLoS ONE* **7**: e32069..

Application to experimental evolution

G_0

Ancestral non-metallicolous population
with a strong genetic diversity

n generations

$ZnSO_4$ contaminated soil

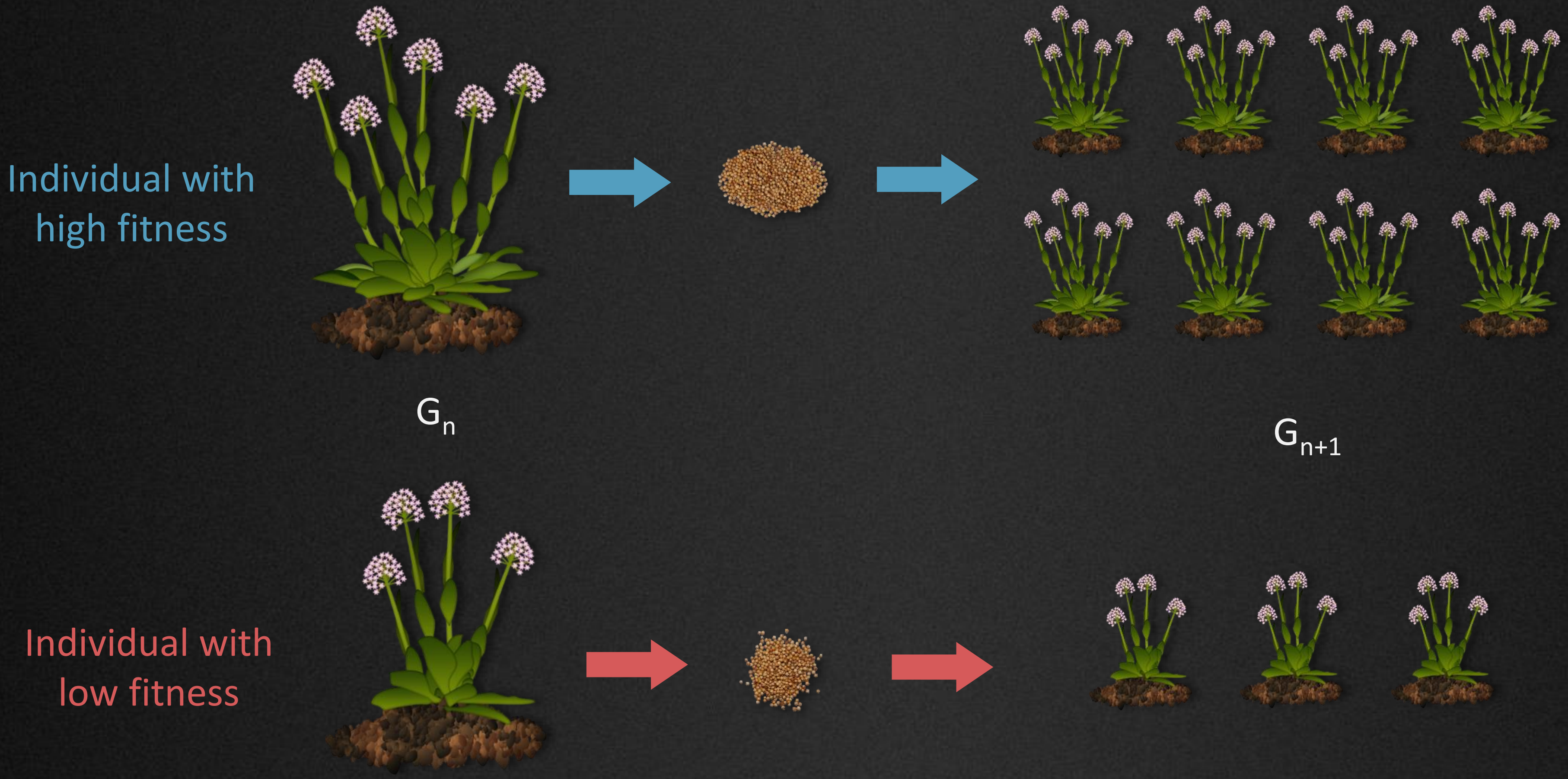
G_n

Derived population with
metallicolous phenotype

How to switch from a generation to another one?

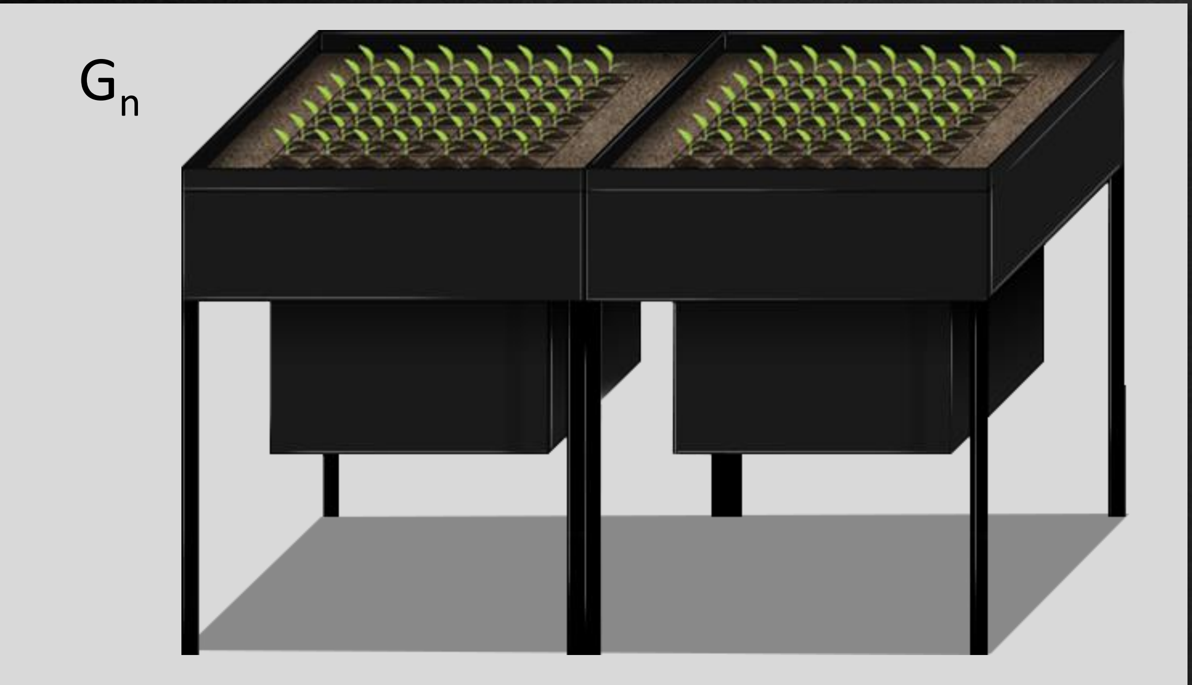
Does zinc represent a suitable selection pressure to cause a change in the population?

Application to experimental evolution



Seeding in proportion to the individual fitness

Application to experimental evolution



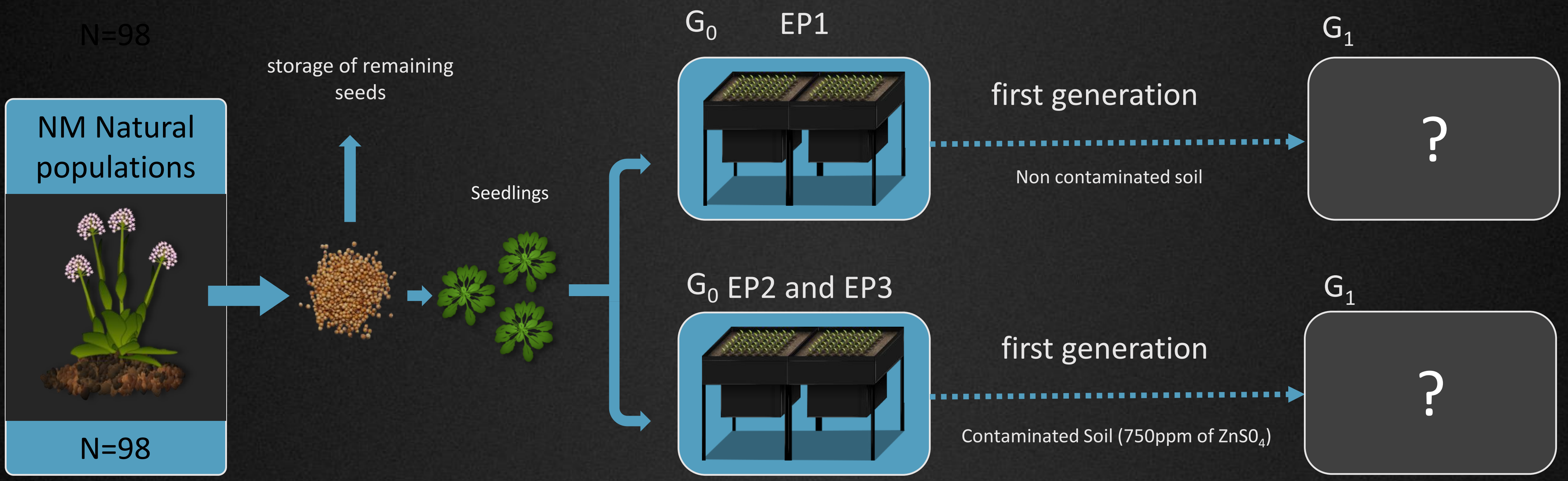
Phenotyping and seed harvest

Relative fitness estimation

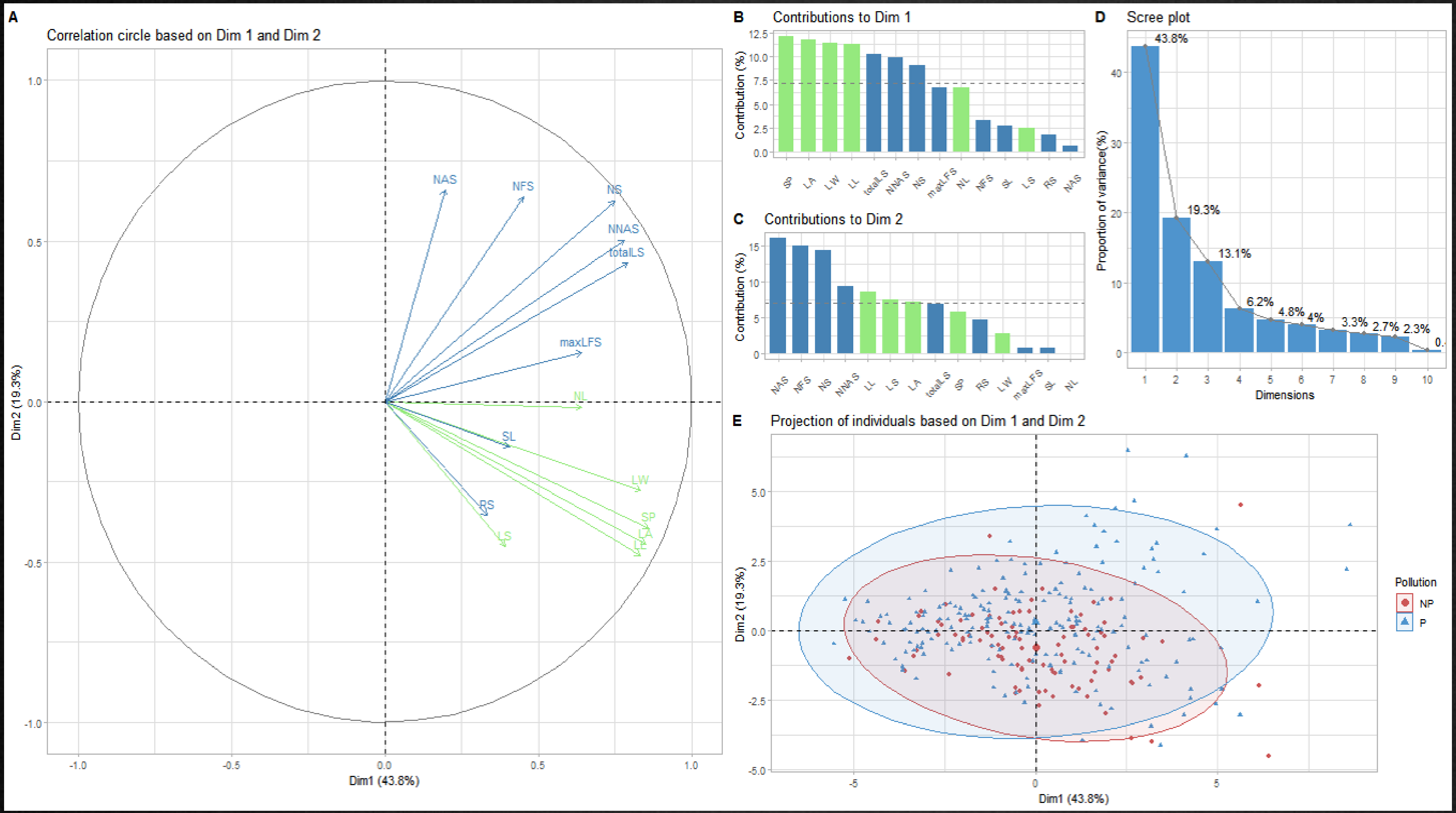
Seedlings

Creation of generation G_{n+1}

Application to experimental evolution

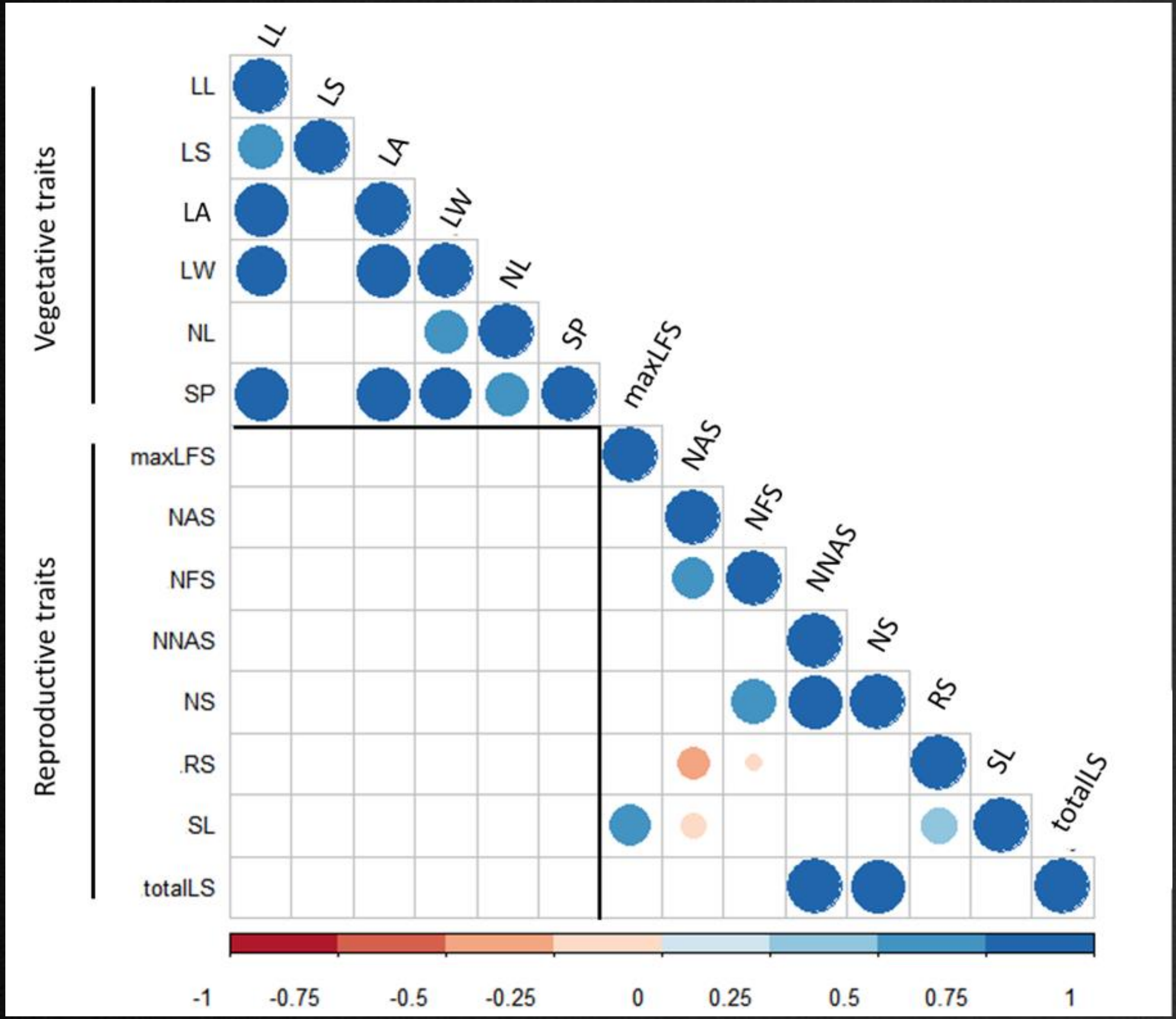


Application to experimental evolution



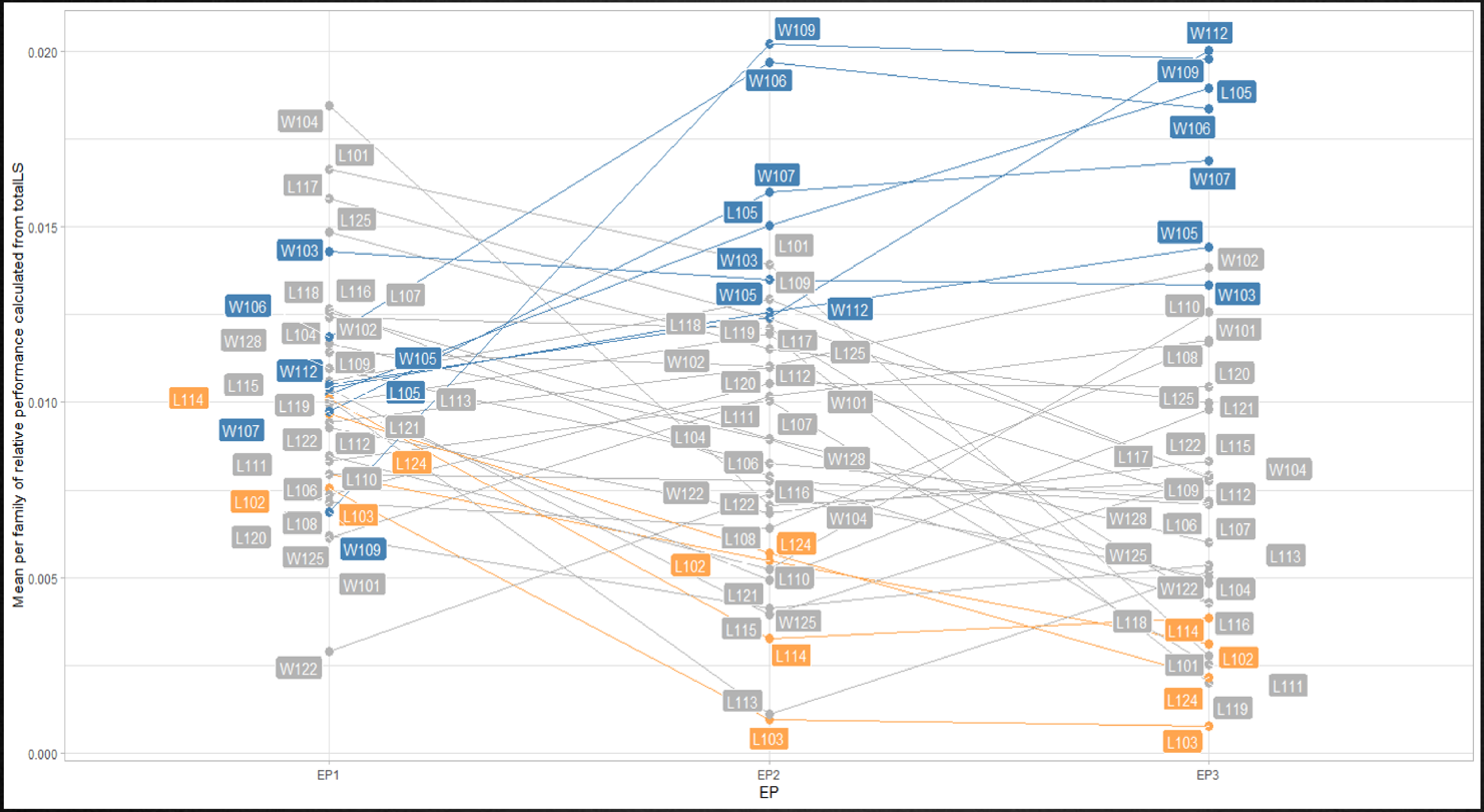
Results of the principal component analysis performed on experimental populations including the correlation circle based on dimension 1 and dimension 2 (A), the variable contributions to dimension 1 (B), the variable contribution to dimension 2 (C), the scree plot of eigenvalues (D), and the projection of individuals based on dimension 1 and dimension 2 according to pollution level (E). P=polluted condition (EP2 and EP3); NP=non-polluted condition (EP1). Vegetative traits: SP=surface of the plant, LL=leaf length, LW=leaf width, LS=leaf shape, LA=leaf area, NL=number of leaves. Reproductive traits: SS=seed set, NFS=number of flower stems, maxLFS=length of the longest stem, NS=number of silicles, NAS=number of aborted silicles, NNAS=number of non-aborted silicles, RS=ratio between NNAS and NAS, SL=silicle length, totalLS=total length of silicles. Ellipse surfaces grouped 90% of individuals for each pollution level. For B and C, the dotted line represents the contribution if all variables contributed equally.

Application to experimental evolution



Correlation matrix among all vegetative and reproductive traits in the experimental populations. The sizes of the circles and the intensities of the colors are proportional to the strength of the correlation. Vegetative traits: SP=surface of the plant, LL=leaf length, LW=leaf width, LS=leaf shape, LA=leaf area, NL=number of leaves. Reproductive traits: SS=seed set, NFS=number of flower stems, maxLFS=length of the longest stem, NS=number of siliques, NAS=number of aborted siliques, NNAS=number of non-aborted siliques, RS=ratio between NNAS and NAS, SL=silique length, totalLS=total length of siliques.

Application to experimental evolution



Rank comparison between relative performances based on the estimated total length of silicles (totalLS) calculated from the mean of each family in EP1, EP2 and EP3. Blue points represent families of the first quartile of the most successful plants present in the two polluted EPs (EP2 and EP3). Yellow points represent families of the last quartile of the less successful plants present in the two polluted EPs (EP2 and EP3). Grey points represent the other families.

Thank you !