![HAL open science logo]

Secondary metabolites have more influence than morphophysiological traits on litter decomposability across genotypes of Arabidopsis thaliana

Elena Kazakou, François Vasseur, Kevin Sartori, Etienne Baron, Nick P Rowe, Denis Vile

▶ To cite this version:

HAL Id: hal-02356210

https://hal.umontpellier.fr/hal-02356210

Submitted on 26 May 2020

Secondary metabolites have more influence than morpho-physiological traits on litter decomposability across genotypes of *Arabidopsis thaliana*.

Elena Kazakou[*1], François Vasseur[*2,3], Kevin Sartori[2], Etienne Baron[2], Nick Rowe[4] and Denis Vile[3]

*These authors equally contributed to this work.

1. Montpellier Supagro, Centre d'Ecologie Fonctionnelle et Evolutive (UMR CEFE), CNRS, Université de Montpellier - Université Paul-Valéry Montpellier - EPHE, Montpellier, 34090, France (https://orcid.org/0000-0001-7188-8367)

2. CEFE, CNRS - Université de Montpellier - Université Paul-Valéry Montpellier - EPHE, Univ Montpellier, Montpellier, 34090, France, (FV :https://orcid.org/0000-0002-0575-6216;KS : https://orcid.org/0000-0001-7364-1341)

3. LEPSE, INRA, Montpellier SupAgro, Univ Montpellier, Montpellier, 34060, France, (https://orcid.org/0000-0002-7948-1462)

4. AMAP, CNRS, INRA, IRD, Univ Montpellier, CIRAD, Montpellier, 34090, France, (https://orcid.org/0000-0003-2680-8545)

* Corresponding author: elena.kazakou@cefe.cnrs.fr; tel : 0033 (0)467613334

Summary

1. Although interspecific variation in plant phenotype is recognized to impact afterlife processes such as litter decomposability, it is still unclear which traits and selection pressures explain these relationships. Examining intraspecific variation is crucial to identify and compare trait effects on decomposability, and investigate the potential role of natural selection.

2. We studied the genetic variability and relationships between decomposability, plant traits typically related to decomposability at species level (morpho-physiological traits), and leaf metabolites among a set of genotypes of *Arabidopsis thaliana* grown under controlled conditions. We also investigated correlations between decomposability and environmental variables at genotypes collection site. We investigated the genetic architecture of decomposability with genome-wide association studies (GWAS).

3. There was large genetic variability in decomposability, which was positively correlated with precipitation. Morpho-physiological traits had a minor effect, while secondary metabolites, especially glucosinolates, were correlated to decomposability. Consistently, GWAS suggest that genes and metabolites related to the composition of cell membranes and envelopes control the variation of decomposability across genotypes.

4. Our study suggests that decomposability varies within species as a result of metabolic adaptation to climate. Our findings in highlight that subtle variations of defence-related metabolites like glucosinolates may strongly influence afterlife processes such as decomposability.

Keywords: *Arabidopsis thaliana*, gene ontology, environment, functional traits, litter decomposability, natural variation, leaf economics spectrum, glucosinolates.

Introduction

Tremendous efforts in trait-based ecology have helped us elucidate the drivers of key ecological processes involved in carbon and nutrient cycling, including litter decomposability (Chapin, 1980; Coûteaux *et al*., 1995; Swift *et al.,* 1998; Cornwell *et al*., 2008; Garnier *et al*., 2006). Phenotypic drivers of litter decomposability have been mainly studied by comparative approaches at the interspecific level, which allow generalizations and predictions (Shipley, 2007; Garnier *et al.*, 2016), but remain limited in explanatory power. It is crucial to take into account intraspecific variation to understand the rules that determine community assembly and ecosystem functioning (Crutsinger *et al.*, 2009; Des Roches *et al.*, 2017). Recent studies showed that there are similar levels of

intraspecific and interspecific trait variations (Albert et *al.*, 2010). Moreover, it has been suggested that genetic diversity within plant populations could alter key ecological processes such as litter decomposability (Wright *et al.* 2017). Exploring the effect of genetic variability on the traits underlying these processes can be a way to reconcile ecological and evolutionary drivers (e.g., Donovan *et al.*, 2011). Here, using a large set of *Arabidopsis thaliana* natural genotypes, we evaluated how much of the variation in litter decomposability was correlated to genetic variation in (*i*) morpho-physiological traits, including leaf functional traits and plant life history traits, and (*ii*) leaf metabolism such as the abundance of secondary metabolites.

Among the different factors expected to impact litter decomposability, i.e. litter mass loss measured in standardized conditions, interspecific variation in leaf structure and physiology has important "after-life effects" on litter quality (Cornelissen *et al.*, 1999; Cornelissen & Thompson, 1997; Santiago, 2007; Parsons & Congdon, 2008). In particular, the trade-off between carbon acquisition and nutrient retention at the leaf level (the so-called 'leaf economics spectrum', LES, Wright *et al.*, 2004) involves a suite of leaf functional traits that explain variation in leaf decomposability across species (Cornwell *et al.* 2008). For instance, specific leaf area (SLA; leaf area divided by leaf dry mass) tends to be negatively associated with high foliar concentrations of structural (e.g. lignin) or chemical compounds that promote protection against herbivores or the abiotic environment (Choong *et al.*, 1992 ; Grime *et al.*, 1997). SLA is related to leaf thickness and tissue density, and is positively correlated with litter decomposability across species (*e.g.*, Cornelissen *et al.*, 1999; Vaieretti *et al.*, 2005; Santiago, 2007; Kurokawa & Nakashizuka, 2008). By contrast, leaf dry matter content (LDMC; ratio leaf dry: fresh mass; (Kazakou *et al.*, 2006; Cortez *et al.*, 2007; Kurokawa & Nakashizuka, 2008) and leaf toughness (Cornelissen & Thompson, 1997; Cornelissen *et al.*, 1999; Kazakou *et al.*, 2009) have a negative effect on litter decomposability. Finally, physical anti-herbivore defence, such as trichome density at the leaf surface, can also affect decomposition and nutrient release (Gallardo & Merino, 1993; Cornelissen & Thompson, 1997). Species with long-lived leaves (high leaf lifespan, LLS) are often characterized by higher concentrations of lignin but lower concentrations of N and P (Coley, 1988). Evidence that the litter decomposability depends on the lignin : N ratio as well as on leaf N and C concentrations (Aerts, 1997 ; Cornelissen, 1996; Cornelissen & Thompson,1997) suggests that there is a link between plant physical defence and litter decomposability (Grime *et al.*, 1996 ; Cornelissen *et al.*, 1999).

As suggested in the review of Chomel *et al.* (2016), the relationships between morpho-physiological traits and litter decomposability are often idiosyncratic, which can be explained by the key role played by secondary metabolites. Several studies have demonstrated that certain secondary metabolites, especially polyphenolics, can limit growth

and activity of decomposers, from microorganisms to macroarthropods (Hättenschwiler & Vitousek 2000; Loranger *et al.* 2002; Chomel *et al.* 2014). Litter decomposability is generally negatively correlated with the concentration of lignin, a group of complex aromatic polymers present in plant cell walls that is recalcitrant to enzymatic degradation and serves as a structural barrier impeding microbial access to labile carbon compounds (Austin & Ballaré, 2010). Most of the studies linking secondary metabolites and decomposability have focused only on lignin, polyphenols and tannins. However, as suggested by Chomel *et al.* (2016), the secondary metabolites present in the leaf and subsequently in the litter should be considered as a key factor influencing decomposer activity. For instance, variation in the abundance and diversity of defence metabolites could also have after-life effect on tissue degradation by decomposers. Nonetheless, no intraspecific study has investigated and compared the respective role of plant morpho-physiological traits, including leaf traits typically related to decomposability at species level, to a large number of secondary metabolites potentially involved in litter decomposability.

A critical step in trait-based ecology is to evaluate the extent of genetic variation within species. Recent research has emphasized the importance of intraspecific variation on ecosystem functioning, including primary productivity (Zhu *et al.*, 2000; Crutsinger *et al.*, 2006), ecosystem stability (Hughes & Stachowicz, 2004; Reusch *et al.*, 2005) and community structure (Violle e*t al.*, 2012). Like plant species, different plant genotypes can vary considerably in the quantity and quality of leaf litter produced, creating genotype-specific differences in rates of decomposition and nutrient release (Driebe & Whitham, 2000; Treseder & Vitousek, 2001; Madritch & Hunter, 2004; 2005; Schweitzer *et al.*, 2005; Silfver *et al.*, 2007). Most of the studies on intraspecific variation in decomposability have focused on tree species (for example Silfver *et al.*, 2007 for *Quercus* and *Alnus* species and Silfver *et al.*, 2015 for *Betula pendula* species) or aquatic species (Lecerf & Chauvet, 2008). Plants have been compared between few genetically distinct genotypes (Treseder & Vitousek, 2001; Jackson *et al.*, 2013), or from hybrids and their parental species (Driebe & Whitham, 2000; Schweitzer *et al*., 2004). For instance, Silfver *et al.* (2007) found that 19 genotypes of *Betula pendula* had substantial genotypic variation in leaf litter mass loss at the early stages of the decomposition process, and that this variation was associated with genotypic variation in herbivore resistance and leaf concentrations of soluble proteins and total nitrogen (N). Hines *et al.* (2013) found that variation in litter chemistry among 12 genotypes of *P. australis* can be as great as, or greater than, variation reported among species (Cornwell *et al.*, 2008) or in

response to environmental variation (Reich & Oleksyn, 2004; Wright *et al.*, 2004; Santiago, 2007). Finally, Lecerf & Chauvet (2008) showed that intraspecific variation in decomposability of senescent leaves of alder (*Alnus glutinosa*) was within a range similar to that reported for interspecific variation among co-occurring riparian plant species in Europe. By constrast, Crustinger *et al.* (2009) found that *Solidago altissima* genotypic effects being much weaker than species-level effects as variation among other goldenrod species in decomposition rate was more than twice that of genetic variation within *S. altissima*. Therefore, the significance of within-species variation is still poorly known (Silfver *et al.,* 2009; 2015), although it is essential to perform intraspecific analysis where phylogenetic correlation do not obscure causation. For instance, using a large number of genotypes covering a wide geographical range would enable quantitative genetic analyses – such as genome-wide association studies (GWAS) – and population genetics analyses, a prerequisite for inferring evolution and adaptation. To date, the genes and molecular pathways that influence litter decomposability still remain unknown.

The model species *Arabidopsis thaliana* is promising to examine the phenotypic and genetic drivers of litter decomposability. Indeed, *A. thaliana* has been widely studied in molecular biology, cell biology and quantitative genetics because of its small stature and short life cycle (*e.g.*, Clark *et al.*, 2006; Platt *et al.*, 2010, Horton *et al*., 2012; Alonso-Blanco *et al*., 2016). The genomic sequencing of natural populations in this species allows performing GWAS to examine the genetic architecture of litter decomposability and identify candidate genes. Combined with its wide distribution across contrasted climates and its important phenotypic variability, *A. thaliana* is also becoming a species of interest for trait-based ecology. For instance, recent studies have shown the same patterns of trait covariations in *A. thaliana* and across species, such as relationships between SLA, LNC, leaf lifespan and photosynthetic rate (Vasseur *et al.*, 2012; Vasseur *et al.,* 2018b). In addition, high-throughput metabolomics has been successfully performed across hundreds of *A. thaliana* genotypes (Wu *et al*., 2018). This generated massive and unprecedented data to investigate the role that leaf metabolites could have on specific ecological processes. For instance, glucosinolates are important secondary metabolites involved in plant defence among species of the Brassicaceae family. In *A. thaliana*, they are notably involved in plant defence against herbivores, bacteria, and fungi (**Kliebenstein *et al*. 2002;** Reichelt *et al.,* 2002)**.** However, their potential role in litter decomposability remains to be elucidated.

In the present study, we hypothesized that litter decomposability is related to the heritable variation of underlying traits, and thus genes. We tested this hypothesis using 211

genotypes of *A. thaliana* and addressed four questions. First, does genetic variation for litter decomposability occurs within a global set of genotypes of *A. thaliana*? Second, at the within-species level, is litter decomposability better explained by morpho-physiological traits or by secondary metabolites? Third, is intraspecific variation in litter decomposability correlated with climate at the genotypes' collection sites? Finally, is litter decomposability heritable and what are the genes involved in this after-life process?

Materials and Methods

Plant material and experimental design

We randomly sampled 211 genotypes from the RegMap collection (Fig. 1, Table S1) (Horton *et al.*, 2012). Seeds used in the present study were provided by Fabrice Roux (Laboratoire des interactions plantes microorganisms LIPM, Auzeville). Plants were grown in three replicates in a semi-controlled greenhouse environment. Seeds were sown on organic soil and stratified at 4 °C for four days. At the emergence of the first two true leaves, plants were transplanted in 300 mL individual pots filled with a 1:1 (v:v) mixture of loamy soil and organic compost (Neuhaus N2). Pots were randomly distributed among three blocks (one replicate per block) that were rotated every day. All pots were watered twice a week. Additional light provided ca. 65 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at plant height. Photoperiod and temperature were kept constant at 12 h day length, and 18/16 °C day/night, respectively.

Litter collection and decomposition assay

Plant litter was collected at the moment of maximum leaf senescence for each genotype. As *A. thaliana* retain dead leaves on the rosette, we cut off leaves that were dead in order to prevent decomposition by fungi when litters were in contact with the soil. Litters were carefully cleaned, then air-dried and stored in the laboratory. The decomposability of each litter sample was assessed using a standard laboratory bioassay in microcosms as described by Wardle *et al.* (2002) (for a detailed description see Supplementary Methods S1).

The percentage of the initial litter mass remaining after incubation (LMR, %) was calculated as: LMR= (Litter mass$_{initial}$ - Litter mass$_{final}$)/Litter mass$_{initial}$ * 100, where Litter mass$_{initial}$ is the dry litter leaf mass at the beginning of incubation and Litter mass$_{final}$ the dry litter leaf mass after the 60 days of incubation in the microcosms.

Measurement of plant morpho-physiological traits

Plant morpho-physiological traits, including leaf functional traits and plant life history traits, were measured using standardized procedures in three replicates per genotype (Pérez - Harguindeguy *et al*., 2013). For each plant, leaf lifespan (LLS) was estimated as the average number of days from leaf emergence to full senescence using daily pictures of one plant replicate per genotype where three consecutive leaves were tracked from emergence to full senescence. One adult, fully-exposed and non-senescent leaf was harvested from each individual (n = 3 per genotype) at bolting stage. Harvested leaves were water-saturated in deionised water at 4 °C for 24 h, scanned for leaf area, measurement (LA, mm^2) and weighed. Specific leaf area (SLA, m^2 kg^{-1}) and leaf dry matter content (LDMC, mg g^{-1}) were calculated as the ratio between leaf area and leaf dry mass, and between leaf dry mass and saturated fresh mass, respectively. Average trichome density (cm^{-2}) was estimated from trichome counts on adaxial and abaxial leaf sides of two 28 mm^2 zones of the harvested leaf. We determined the leaf nitrogen content (LNC, %) on one leaf replicate per genotype with an elemental analyzer (model EA 1108; Carlo Erba Instruments, Milan, Italy). According to Onoda *et al.* (2011) an approximation of the structural resistance to shearing (LRS, J m^{-1}) was measured using a protocol similar to that of Ang *et al*. (2008) using a single blade cutting device mounted on a portable Instron inspec 2200, instrumented device that measured the force (N) and distance of the downward cutting blade, (Instron Engineering Corp., Canton, MA, USA). Leaves were cut in the middle position of the leaf length, from the leaf margin up to but not including the midrib. The horizontal distance cut was calculated via the vertical displacement and the angle of the blade set to 30° from the horizontal. Flowering time (FT) was measured at bolting stage (first emergence of the floral bud) and was included because it represents a major trait associated with plant life history and resource-use strategies (Vasseur *et al*., 2012, 2018a, 2018b).

Leaf metabolites dataset

Quantitative variation in untargeted metabolites – i.e. all measurable metabolites – has been measured recently across 309 genotypes by Wu *et al.* (2018). Wu and colleagues reported variation for 4,182 metabolites in non-stressing conditions. 91 genotypes were common to Wu *et al*. (2018) and the present study. Due to missing data, only 2,132 metabolites were retained for subsequent analyses here. Four genotypes among the 91 had missing data for one or several metabolites, which were inputted with the 'missMDA' package in R. We first investigated whether there were correlations between decomposition rate and metabolites

over all metabolites measured in Wu *et al.* (Fig. 3C, D, see later). Then, we went further by looking more specifically to a subset of 103 metabolites for which functions were identified in Wu *et al*. (2018) (Fig. 4, see later). In doing so, our approach was voluntarily not oriented toward a specific class of metabolites, but rather to test if there is an effect of metabolites on decomposability over the broadest range of metabolites that we could use. To compare the role of different classes of metabolites, we used the set of 103 identified metabolites from Wu *et al.* (2018), and investigated the correlations between each metabolite and LMR. Spearman's coefficients of correlation were reported, after grouping by metabolite class (amino acid - amine, coenzyme, flavonoid, glucoside - sugar, glucosinolate, nucleoside – nucleotide and their derivative, organic acid, and phenylpropanoid). For convenience, amines and amino acids were treated together, as well as sugars and glucoside, and nucleotide, nucleoside and their derivatives. Only the classes containing at least three identified metabolites were kept for the analysis.

Statistical analyses

For each trait, genotypic means (trait mean by genotype) were estimated from linear models (package 'lsmeans'). Principal Component Analyses (PCA) were performed separately on morpho-physiological traits (morpho-physiological PCA), metabolites (metabolomic PCA) and climate variables (climatic PCA) using the 'ade4' package. The morpho-physiological PCA was performed across the 91 genotypes with both morpho-physiological traits and metabolites, using leaf area, leaf resistance to shearing (LRS), SLA, LDMC, leaf lifespan (LLS), bolting time (FT), nitrogen concentration (LNC) and trichome density. Metabolomic PCA was also performed on the same 91 genotypes, using the 2,132 untargeted metabolites measured by Wu *et al*. (2018). Climatic PCA was also perfomed on the 91 accessions, using the climate variables at the collection points of the genotypes extracted from WorldClim database (http://www.worldclim.org/bioclim). For each PCA, we retained all principal components (PCs) that explain more than 5% of total genetic variability (Fig. S2) for subsequent analyses.

To quantify the respective role of functional traits and metabolites on litter decomposability, we performed a multivariate random forest model, using the 'randomForest' package in R, which allows model classification based on Breiman's random forest algorithm (Breiman, 2001). All PCs were scaled (standard deviation = 1) and centered (mean = 0) prior to analysis. In addition, and following previous approaches (*e.g.*, Shantz & Burkepile, 2014; Barnagaud *et al.,* 2013; Leonard *et al.*, 2014; Welti & Joern, 2014), we

fitted with linear regressions all possible combinations of explanatory variables (*i.e.* morpho-physiological PCs and metabolomic PCs) nested within the full model, using the *dredge* function in the 'MuMIn' package (Barton, 2012). Then, we plotted the averaged coefficients fitted for each explanatory variable across all possible models. This approach is powerful, as it helps to minimize loss of information contained in the data (Schielzeth, 2010). Moreover, since predictor variables (*i.e.* PCs) were rescaled before analysis, the distribution of these fixed effects can be interpreted as effect sizes. That is, a coefficient with value +1 indicates that a 1 SD increase in the predictor variables (*i.e.* PCs) directly causes a 1 SD increase in the response variable (*i.e.* LMR), after accounting for any other predictor variables (Blonder *et al.*, 2018). We followed the same procedure for climate PCs. All analyses were performed using R 3.15 (R Core Team, version 3.5.3, 2019).

Quantitative genetics analysis

Classical ('monogenic') GWAS (genome-wide association studies) were performed on 214,050 biallelic SNPs from the publicly available RegMap genetic panel (Horton *et al*., 2012). GWAS exploits natural genotypic variation and enables the analysis of significant associations between hundreds of thousands of single-nucleotide polymorphisms (SNPs) and specific phenotypes (Horton *et al*., 2012). GWAS was carried out using EMMAX (Kang *et al*., 2010) implemented in the online platform easyGWAS (https://easygwas.ethz.ch/; Grimm *et al.*, 2017), with minimum allele frequency at each SNP above 5%, and which including the two first axes of a PCA performed on SNPs as covariates to take into account population structure in *A. thaliana*. Significance threshold that considers multiple testing correction was determined with the Bonferroni method ($\alpha = 0.05$ or 0.1).

Polygenic GWAS were performed with Bayesian Sparse Linear Mixed model (BSLMM) implemented in EMMA (Zhou *et al.*, 2013), which accommodates both poly- and oligogenic architectures. Chip heritability (h^2) was calculated from BSLMM models as the sum of phenotypic variance explained by all SNPs, assuming genetic additivity. Given the importance of metabolome PC3, we performed BSLMM and GO analysis to examine the biological functions associated with this axis. By contrast with monogenic GWAS, BSLMM models two effect hyperparameters, a basal effect, alpha, that captures the fact that many SNPs contribute to the phenotype, and an extra effect, beta, which captures the stronger effect of only a subset of SNPs. We summed alpha and beta to estimate total SNP effect and investigate the correlation between SNP effects on LMR and metabolome PC3, respectively. We then used the 1% top-SNPs (*i.e.* 0.5% at the lower tail and 0.5% at the upper tail) of the

distribution of SNP effect to identify the genes that contributed most to the phenotypic effect. Gene names were extracted from SNPs position using gene annotation along the *A. thaliana* genome from the TAIR10 GFF3 file. This resulted in 1,910 top-genes in total, for LMR, and 3,398 top-genes for metabolome third PC axis. We then performed Gene Ontology (GO) analysis using the AgriGO (Du *et al.*, 2010) and REVIGO online tools to visualize significant enrichment in GO categories (Supek *et al.*, 2011).

Results

Genetic variability in litter decomposability and morpho-physiological traits

We found a marked variation in litter decomposability among the 211 genotypes of *A. thaliana* (Fig. 2): litter mass remaining (LMR) ranged from 21 to 57 %. Significant variability was also found in morpho-physiological traits (Fig S1): trichome density was the most variable trait (25-fold variation; from 0 to 24 trichomes per cm^2, Fig. S1H). LES traits were also very variable, notably leaf dry matter content (LDMC) (5.4-fold variation between 65 and 351 mg g^{-1}, Fig. S1C) and leaf nitrogen content (LNC) (20.8-fold variation between 0.4% and 8%, Fig. S1F), while specific leaf area (SLA) and leaf life span (LLS) varied 5 and 3.5 times (from 12.3 to 91.3 m^2 kg^{-1} and from 15 to 53.5 d, respectively) (Fig. S1D and S1G). In addition, flowering time and leaf area varied between 25 and 113 days, and between 104 and 811 mm^2, respectively (Fig. S1A and S1B).

Comparing the effect of plant morpho-physiological traits and metabolites on litter decomposability

The two first PCs of the morpho-physiological PCA explained together 80% of the multivariate genetic variability (62% and 18% for PC1 and PC2, respectively; Fig. 3A), while PC3 and PC4 explained 10% and 5%, respectively, of total genetic variability. PC1 represented the leaf economics spectrum: positive PC1 values were associated with acquisitive strategies characterized by high specific leaf area (SLA) and high leaf nitrogen content (LNC), but short lifespan, low leaf dry matter content (LDMC) and low structural resistance. Inversely, negative PC1 values were associated with conservative strategies characterized by opposite leaf trait values. PC2 was mainly explained by variation in leaf area (Fig. 3A). The four first PCs of the metabolomic PCA together explained 36% of metabolomic variability (PC1: 13%, PC2: 11%, PC3: 7%, PC4: 5%; Fig. 3B).

We then used random forest models and multiple model evaluation to compare the respective role of morpho-physiological trait variation and metabolomic variation on litter

mass remaining (LMR). Models were performed on the 91 genotypes subset, using the first five PCs of both morpho-physiological traits and metabolomic PCA. Random forest model indicated that PC3 of the metabolomic PCA was the main contributor of litter mass remaining (LMR), followed by the second PCA axis of morpho-physiological traits (Fig. 3C). By comparison, intraspecific variations related to the leaf economics spectrum and structural resistance (morpho-physiological PC1) had only small effects on litter mass remaining (LMR) variability among genotypes, even less than morpho-physiological PC4 and metabolomic PC2 (Fig. 3C). Consistently, multiple model evaluation suggested that metabolomic PC3 had the strongest effect (+40%) on litter mass remaining (LMR), while morpho-physiological PC1 had no significant effect (Fig. 3D). Multiple model evaluation also suggested that morpho-physiological PC2 had a significant negative effect on litter mass remaining (LMR), suggesting that bigger leaves had higher decomposability (*i.e.* lower LMR).

Using a set of 103 metabolites identified from Wu *et al.* (2018), we explored the role of different classes of metabolites on leaf decomposability (Fig. 3; Table S2). Results suggest that glucosinolates, a group of secondary metabolites involved in plant defence in Brassicaceae like *A. thaliana*, also had globally a negative effect on decomposability, as illustrated by the positive correlations between individual glucosinolates and LMR (14 glucosinolates out of 32 were significantly and positively correlated with LMR, $P < 0.05$; Table S2). In addition, amino acids composition also had a notable effect on decomposability, with some amino acids being negatively correlated with LMR (*e.g.*, L-glutamic acid, indole-3-lactic acid, homoserine; Table S2) and others being positively correlated (*e.g*., L-tryptophan, methionine, valine; Table S2). The same result was found for flavonoids, nucleotides/nucleosides and organic acids (Fig. 4). Finally, phenylpropanoids were on average negatively correlated with LMR, although none of the correlation with the underlying metabolites was significant ($P > 0.05$; Table S2).

Effect of climate of origin on litter decomposabiilty

The PCA on the 19 climate variables that describe the environment of origin of each genotype (Fig. 5A) showed that PC1 was mainly associated to temperature variables (with those related to winter temperature as top contributors), while with PC2 mainly accounted for precipitation variables (with those related to summer precipitation as top contributors). Together, PC1 and PC2 accounted for 62% of variability (PC1 = 35%, PC2 = 27%). Rainfall-related variables at collection sites (PC2) had a small but significant effect on litter mass

remaining (LMR (Fig. 5B), suggesting that adaptation to water availability leads to variation in leaf properties that can alter decomposability. However, the lack of correlation between climatic PC2 and metabolomic PC3 ($r^2 = 0.01$, $P = 0.30$) suggests that the changes in leaf properties associated with precipitation are independent from the metabolites identified here as associated with litter decomposability (metabolomic PC3). Finally, morpho-physiological PC1 was negatively correlated to climatic PC1, relating species with high acquisitive strategies to warmer temperatures than species with conservative strategies (Table S3).

Genetic bases of litter decomposability

Chip heritability of litter mass remaining, a measure of narrow-sense heritability (h^2), was estimated to 0.25 with BSLMM. This indicated a substantial genetic control of litter decomposability, presumably because the underlying determinants of litter decomposability (*e.g.*, leaf metabolome) have a strong genetic basis. No SNP was significantly associated with LMR along the genome after multiple testing correction (Fig. 6A), which can be explained by a large number of genes contributing to the phenotype, each with small effect – *i.e.* a 'polygenic' architecture – rather than few genes with strong effect on the phenotype, as classically tested under the monogenic GWAS. We found that the 1,910 top-genes involved in LMR were indeed significantly enriched for specific cellular components such as proteins localized in plasma membrane and chloroplast envelope (Fig. 6B). In addition, we also found a significant enrichment in the biological processes related to post-embryonic development, post-translational protein modification and transport (Fig. S3C). Finally, the molecular functions of genes involved in litter decomposability were significantly enriched for functions related to ATP binding and ATPase activity, as well as transmembrane transporter activity (Fig. S3E).

Given the importance of metabolomic PC3, we performed BSLMM and GO analysis to examine the biological functions associated with this axis. Interestingly, GO analysis of the 1% top-genes revealed a significant enrichment for proteins localized in plasma membrane and chloroplast envelope (Fig. 6C), similarly to what has been found for LMR (Fig. 6B). Metabolomic PC3 was also significantly enriched for 1,3-beta-D-glucan, a key polymer of cell wall composition. Moreover, we estimated with BSLMM the SNP effects on each individual variable (*i.e.* metabolomic PC3 and LMR), and inspected their correlation (Fig. 6D). As expected many SNPs had a null effect on each variable, generating a cross along the zero axes when plotting the relationship between SNP effects on each variable in Fig. 6D. However, we found that SNP effects on metabolomic PC3 were significantly and positively

correlated with SNP effects on LMR ($r = 0.05$, $P < 0.001$; Fig. 6D), which suggests that differences in litter decomposability across *A. thaliana* genotypes are at least in part explained by variation in metabolomic PC3, such as the chemical properties of plasma membranes and cell wall.

Discussion

In this study, we examined the drivers of litter decomposability within species by comparing the role of plant morpho-physiological traits and leaf metabolites among *A. thaliana* genotypes. Our results showed that litter decomposability vary by 2.7-fold and is partly heritable ($h^2 = 0.25$). This heritable variation of litter decomposability can be attributed to adaptation to different precipitations across the distribution range of *A. thaliana*, and it is mainly due to genetic variations in leaf metabolites rather than the genetic variations in plant morpho-physiological traits typically found at the species level.

The intraspecific range of variation in litter decomposability observed in this study is comparable to the variability found in several interspecific analyses. For example, litter mass remaining (LMR) from several herbaceous species measured in similar conditions (leaf litter incubated in microcosms for 90 days with standardized soil and controlled temperature and soil humidity) ranged from 30% for *Crepis foetida* to 92% for *Carex humilis* (3-fold) (data from Kazakou *et al.*, 2007 and Bumb *et al.*, 2018). Previous studies at the interspecific level have demonstrated that litter decomposability is partly controlled by tissue nutrient concentration and by the density of structural material in the leaf (Cornelissen *et al.*, 1999; Wardle *et al.*, 2002, Cornwell *et al*., 2008). For instance, nutrient concentration in green leaves, such as nitrogen, carbon or phosphorus content, are likely to be linked with litter nitrogen content or with the litter lignin:nitrogen ratio (Wright *et al.*, 2004). Traits such as leaf dry matter content (LDMC) might also reflect structural support and defence against herbivores, which makes them good predictors of litter quality and thus litter decomposability across species (Cornelissen and Thompson, 1997; Kazakou *et al*., 2006). Indeed, positive correlations were observed, always at the interspecific level, between leaf dry matter content (LDMC) and lignin content (Cornelissen *et al.*, 2004; Kazakou *et al.,* 2006; Quested *et al.,* 2007; Fortunel *et al.,* 2009). Together, these traits are part of the leaf economics spectrum that describes resource-use strategies of living leaves (Wright *et al*., 2004). We thus tested the hypothesis that, within species, litter decomposability can be predicted by a set of leaf traits in a manner similar to what has been observed across species, such as traits related to the leaf economics spectrum. In particular, we expected a positive relationship between resource-

acquisitive strategies and litter decomposability. Our results showed that the leaf economics trade-off was indeed reflected by leaf trait covariations across the set of genotypes studied, in a similar way as at the interspecific level. This variation was captured by morpho-physiological PC1: rapid acquisition of resources is generally correlated with high specific leaf area (SLA) and leaf nitrogen content (LNC), while high leaf dry matter content (LDMC), high leaf resistance to shearing (LRS) and long leaf life span (LLS) reflect a resource conservation strategy. Interestingly, both flowering time and trichome density were negatively correlated with these traits, suggesting that late-flowering plants have conservative strategies with abundant trichomes at the leaf surface. This relationship is consistent with the analysis of ecological strategies among *A. thaliana* genotypes (Vasseur *et al.*, 2018). However, despite the large range of variation in functional leaf traits, our initial hypothesis was not supported by our analyses. Neither particular leaf traits nor morpho-physiological PC1 explained the intraspecific variation in litter decomposability in *A. thaliana*.

Secondary metabolites could also impact litter decomposability and therefore nutrient turnover. For example, numerous studies have pointed out the role of secondary metabolites (lignin, cellulose, hemicellulose) as regulating factors of litter decomposability (Berg, 2000), but the role of secondary metabolites still remain largely underexplored. Our results showed that a large number of metabolites, together grouped along metabolomic PC3, was the main contributor of the variation of litter mass remaining (LMR) in *A. thaliana*. Furthermore, genetic analysis showed that the top-genes associated with litter mass remaining (LMR) variation between genotypes are mainly localized in cell and organelle envelopes. Interestingly, the same enrichment was observed for the genes associated with metabolomic PC3, and SNP effects measured for litter mass remaining (LMR) and metabolomic PC3 were significantly correlated. Thus, litter decomposition in *A. thaliana* seems to be mainly driven by the abundance and diversity of metabolites, notably those related to the chemical composition of cell and organelle envelopes. In addition, glucosinolates were also negatively with the rate of leaf decomposition across genotypes pointing the role of defence metabolites in after-life effects. However, we have to notice that our results were based on the secondary metabolites identified by Wu *et al.* (2018) and other unidentified metabolites may play an important role to the decomposition process. The remarkable diversity of secondary compounds that have been quantified from the model plant *A. thaliana*, present tremendous challenges for the study and interpretation of secondary metabolism.

We found that intraspecific variation in litter decomposability is shaped by numerous genes along the genome, each with relatively weak effect (i.e. a polygenic architecture). Monogenic GWAS did not reveal any significant association, presumably because no gene had an effect strong enough

to reach significance threshold after multiple testing correction. This polygenic architecture of leaf decomposability is consistent with a prominent role of secondary metabolites, which are numerous and diverse, and encoded by many genes. We also found a positive correlation between climatic PC2 and litter mass remaining (LMR). Hence, drier environments might have favored genotypes with higher LMR (i.e. lower decomposability). This positive relationship between precipitation and litter decomposability corroborates interspecific findings where leaves with recalcitrant molecules are selected in mesic conditions (Quested, 2008; Cornwell *et al.*, 2008). A complicating factor is that the temperature and water sensitivity of litter decomposability is also dependent on the quality of the carbon compounds in litter: litter enriched in C-based secondary compounds (i.e. low quality) is more sensitive to temperature and precipitation change than litter with high amounts of-quality C compounds (Fierer *et al.*, 2005). However, our analysis suggests that variation in litter mass remaining associated with adaptation to water availability is independent of the class of metabolites involved in decomposition, as climatic PC2 and metabolomic PC3 were not correlated. A possible explanation is that adaptation to water availability relies on diverse metabolites that were not associated with a specific metabolomic PC, and not with the axis related to cell and organelle envelopes (PC3) in particular. This relation is consistent with recent findings that showed the polygenic architecture of drought adaptation in *A. thaliana* (Exposito-Alonso *et al.*, 2018). Together, our results suggested that selection for resistance to specific stresses during plant life can have after-life effects on important ecological processes such as microbial activity and nutrient cycling. Further studies will be required to identify precisely the metabolites having an after-life effect.

Conclusion

We find a large genetic variability in litter decomposability as well as in morpho-physiological traits, but they were not correlated with each other. By contrast, metabolites located in cell membranes and envelopes played a significant role in litter decomposability. In addition, metabolites related to plant defence, such as glucosinolates, were also negatively correlated with decomposability. Thus, our results illustrated the prominent effect of secondary metabolites on after-life processes at the intraspecific level. Further explorations of the associations between decomposability and secondary metabolites (and especially glucosinolates) are needed, as well as the genes and physiological mechanisms that explain the variation in litter decomposability along environmental gradients. In this perspective, manipulating specific genes related to glucosinolates biosynthesis, for instance using mutants and/or introgression lines, offers a promising avenue to confirm the role of these metabolites in litter decomposability. Moreover, using reciprocal transplants of different genotypes

along specific environmental gradients (related to temperature, precipitation and/or nutrient availability), to measure changes in metabolomic profiles and decomposition rate, would allow teasing apart the effects of different environmental conditions on litter decomposability and its metabolic drivers.

Authors contributions

EK, FV, KS, EB and DV planned and designed the research. EK, FV, KS, EB, NR performed experiments, conducted fieldwork, analysed data. EK and FV wrote the manuscript. EK and FV contributed equally to this work.

References

Albert CH, Thuiller W, Yoccoz NG, Douzet R, Aubert S, Lavorel S. 2010.

A multi-trait approach reveals the structure and the relative importance of intra- vs. interspecific variability in plant traits. *Functional Ecology* **24**: 1192–1201.

Alonso-Blanco C, Andrade J, Becker C, Bemm F, Bergelson J, Borgwardt KM, Cao J, Chae E, Dezwaan TM, Ding W et al. 2016. 1,135 genomes reveal the global pattern of polymorphism in *Arabidopsis thaliana*. *Cell* **166 (2)**: 481-491.

Ang KY, Lucas PW, Tan HT. 2008. Novel way of measuring the fracture toughness of leaves and other thin films using a single inclined razor blade. *New Phytologist* **177**: 830-837.

Austin AT, Ballaré CL. 2010. Dual role of lignin in plant litter decomposition in terrestrial ecosystems. *Proceedings of the National Academy of Sciences of the United States of America* **107 (10)**: 4618-4622.

Barnagaud J, Barbaro L, Hampe A, Jiguet F, Archaux F. 2013. Species' thermal preferences affect forest bird communities along landscape and local scale habitat gradients. *Ecography* **36:** 1218-1226.

Barton K. 2016. MuMIn: Multi-Model Inference. R package version 1.15.6. https://CRAN.R-project.org/package=MuMIn

Berg B. 2000. Litter decomposition and organic matter turnover in northern forest soils. *Forest Ecology and Management.* **133**:13-22.

Blonder B, Salinas N, Patrick Bentley L, Shenkin A, Orlando P, Porroa C, Valdez Y, Tejeira Boza, Espinoza TE, Goldsmith GR, Enrico L, Martin R, Asner GP, Díaz S, Enquist BJ, Malhi Y 2018. Structural and defensive roles of angiosperm leaf venation network reticulation across an Andes–Amazon elevation gradient. *Journal of Ecol*ogy **106:** 1683–1699.

Breiman L. 2001. Random Forests, Machine Learning **45 (1),** 5-32.

Bumb I, Garnier E, Coq S, Nahmani J, Del Rey Granado M, Gimenez O, Kazakou E. 2018. Traits determining the digestibility–decomposability relationships in species from Mediterranean rangelands. *Annals of Botany* **121 (3)**: 459–469.

Burnham KP, Anderson DR. 2002. *Model Selection and Multimodel Inference: A Practical Information‑Theoretic Approach*, 2nd edn. Springer, Berlin.

Calenge F, Saliba-Colombani V, Mahieu S, Loudet O, Vedele FD, Krapp A. 2006. Natural variation for carbohydrate content in *Arabidopsis*. Interaction with complex traits dissected by quantitative genetics. *Plant Physiology* **141**: 1630-1643.

Chapin FS. 1980. The Mineral Nutrition of Wild Plants. *Annual Review of Ecology and Systematics* **11(1)**: 233-260.

Clark RM, Schweikert G, Toomajian C, Ossowski S, Zeller G, Shinn P, Warthmann N, Hu TT, Fu G, Hinds DA et al. 2007. Common sequence polymorphisms shaping genetic diversity in *Arabidopsis thaliana*. *Science* **317**: 338–342.

Cornelissen JHC, Thompson K. 1997. Functional leaf attributes predict litter decomposition rate in herbaceous plants. *New Phytologist* **135 (1)**: 109–14.

Cornelissen JHC, Perez-Harguindeguy N, Diaz, S, Grime JP, Marzano B, Cabido M, Vendramini F, Cerabolini B. 1999. Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two Continents. *New Phytologist* **143 (1):** 191–200.

Cornwell W, Cornelissen JHC, Amatangelo K, Dorrepaal E, Eviner VT, Godoy O, Hobbie SE, Hoorens B, Kurakawa H, Pérez-Harguideguy N et al. 2008. Plant species

traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology letters* **11(10):** 1065–71.

Cortez J, Garnier E, Pérez-Harguindeguy N, Debussche M, Gillon D, Perez-Harguindeguy N. 2007. Plant traits, litter quality and decomposition in a Mediterranean old-field succession. *Plant and Soil* **296 (1–2)**: 19–34.

Coûteaux MM, Bottner P, Björn B. 1995. Litter Decomposition, Climate and Liter Quality. *Trends in Ecology & Evolution* **10 (2)**: 63-6.

Crutsinger GM, Collins MD, James A, Fordyce JA, Gompert Z, Nice CC, Nathan JS. 2006. Plant Genotypic Diversity Predicts Community Structure and Governs an Ecosystem Process. *Science* **313 (5789):** 966–68.

Crutsinger GM, Sanders NJ, Classen AT. 2009. Comparing intra- and inter-specific effects on litter decomposition in an old-field ecosystem. *Basic and Applied Ecology* **10(6):** 535–43.

D'Auria JC, Gershenzon J. 2005. The secondary metabolism of *Arabidopsis thaliana*: growing like a weed. *Current Opinion Plant Biology* **8**: 308-316.

Des Roches S, Post D, Turley N, Bailey K, Hendry J, Kinnison A, Schweitzer M, Palkovacs EP. 2017. The ecological importance of intraspecific variation. *Nature Ecology & Evolution* **2(1)**: 57-64.

Donovan LA, Maherali H, Caruso CM, Huber H, de Kroon H. 2011. The Evolution of the Worldwide Leaf Economics Spectrum. *Trends in Ecology and Evolution* **26 (2)**: 88-95.

Driebe EM, Whitham TG. 2000. Cottonwood hybridization affects tannin and nitrogen content of leaf litter and alters decomposition. *Oecologia* **123 (1)**: 99–107.

Du Z, Zhou X, Ling Y, Zhang Z, Su Z. 2010. agriGO: a GO analysis toolkit for the agricultural community. *Nucleic acids research* **38**.**suppl_2**: W64-W70.

Hines J, Hammrich A, Steiner D, M. O. Gessner GO. 2013. A field facility to simulate climate warming and increased nutrient supply in shallow aquatic ecosystems. *Oecologia* **173**: 1169–1178.

Horton MW, Hancock AM, Huang YS, Toomajian C, Atwell S, Auton A, Muliyati NW, Platt A, Sperone FG, Vilhjálmsson BJ et al. 2012. Genome-wide patterns of genetic variation in worldwide *Arabidopsis thaliana* accessions from the RegMap panel. *Nature Genetics* **44**: 212–216.

Hughes AR, Stachowicz JJ. 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences* **101 (24)**: 8998–9002.

Exposito-Alonso M, Becker C, Schuenemann VJ, Reiter E, Setzer C, Slovak R, Vrachi B, Hagmann J, Grimm DG, Chen J et al. 2018. The rate and potential relevance of new mutations in a colonizing plant lineage. *PLoS Genetics*. **14 (2)**: e1007155.

Fierer N, Craine JM, McLauchlan K Schimel JP. 2005. Litter quality and the temperature sensitivity of decomposition. *Ecology* **86**: 320-326.

Gallardo A, Merino J. 1993. Leaf decomposition in two Mediterranean ecosystems of Southwest Spain: influence of substrate quality. *Ecology* **74**: 152-161.

Garnier E, Navas ML, Grigulis K. 2016. *Plant Functional Diversity. Organism Traits, Community Structure and Ecosystem Properties*. Oxford UK: Oxford University Press.

Grimm DG, Roqueiro D, Salomé PA, Kleeberger S, Greshake B, Zhu W, Liu C, Lippert C, Stegle O, Schölkopf B et al. 2017. 'easy GWAS': Cloud based platform for comparing the results of genome-wide association studies. *Plant Cell* **29 (1)**: 5-19.

Jackson BG, Peltzer DA, Wardle DA. 2013. The within-species leaf economic spectrum does not predict leaf litter decomposability at either the within-species or whole community levels. *Journal of Ecology* **101 (6)**: 1409–19.

Kang HM, Sul JH, Service SK, Zaitlen NA, Kong S, Freimer NB, Sabatti C, Eskinet E. 2010. Variance component model to account for sample structure in genome-wide association studies. *Nature Genetics.* **42**: 348–354.

Kazakou E, Vile D, Shipley B, Gallet C, Garnier E. 2006. Co-variations in litter decomposition, leaf traits and plant growth in species from a Mediterranean old-field succession. *Functional Ecology* **20**: 21–30.

Kazakou E, Violle C, Roumet C, Pintor C, Gimenez O, Garnier E. 2009. Litter quality and decomposability of species from a Mediterranean succession depend on leaf traits but not on nitrogen supply. *Annals of Botany* **104**: 1151–116.

Kerwin R, Feusier J, Corwin J, Rubin M, Lin C, Muok A, Larson B, Li B, Joseph B, Francisco M et al. 2015. Natural genetic variation in *Arabidopsis thaliana* defence metabolism genes modulates field fitness. *eLife* **4:** e05604, 2015.

Kliebenstein D, Pedersen D, Barker B, Mitchell-Olds T. 2002. Comparative analysis of quantitative trait loci controlling glucosinolates, myrosinase and insect resistance in *Arabidopsis thaliana*. *Genetics* **161**: 325–332.

Kurokawa H, Nakashizuka T. 2008. Leaf herbivory and decomposibility in a Malaysian tropical rain forest. *Ecology* **89 (9)**: 2645–56.

Lecerf A, Chauvet E. 2008. Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. *Basic and Applied Ecology* **9 (5)**: 598–605.

Leonard SWJ, Bennett AF, Clarke MF. 2014. Determinants of the occurrence of unburnt forest patches: Potential biotic refuges within a large, intense wildfire in south-eastern Australia. *Forest Ecology and Management* **314:** 85-93.

Madritch MD, Hunter MD. 2004. Phenotypic diversity and litter chemistry affect nutrient dynamics during litter decomposition in a two species mix. *Oikos* **105**: 125–31.

Parsons S, Congdon R. 2008. Plant litter decomposition and nutrient cycling in North Queensland tropical rain-forest communities of differing successional status. *Journal of Tropical Ecology* **24 (3):** 317–27.

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE et al. 2013. New handbook for standardize measurement of plant functional traits worldwide. *Australian Journal of Botany*. **61**: 167-234.

Platt A, Horton M, Huang YS, Li Y, Anastasio AE, Mulyati NW, Ågren J, Bossdorf O, Byers D, Donohue K et al. (2010) The Scale of Population Structure in *Arabidopsis thaliana*. *PLoS Genet* **6 (2)**: e1000843.

Quested HM, Cornelissen JHC, Press MC, Callaghan TV, Aerts R, Trosien F, Riemann P, Gwynn-Jones D, Kondratchuk A, Jonasson SE. 2003. Decomposition of sub-arctic plants with differing nitrogen economies: a functional role for hemiparasites. *Ecology* **84:** 3209–3221.

R Core Team 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reichelt M, Brown PD, Schneider B, Oldham NJ, Stauber E, Tokuhisa J, Kliebenstein DJ, Mitchell-Olds T, Gershenzon J, 2002. Benzoic acid glucosinolate esters and other glucosinolates from Arabidopsis thaliana. *Phytochemistry* **59**: 663–671.

Reusch TBH, Ehlers A, Hämmerli A, Worm B. 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences* **102 (8):** 2826–31.

Santiago LS. 2007. Extending the leaf economics spectrum to decomposition: evidence from a tropical forest. *Ecology* **88 (5)**: 1126–31.

Schielzeth H. 2010. Simple Means to Improve the Interpretability of Regression Coefficients. *Methods in Ecology and Evolution* **1 (2):** 103–13.

Schweitzer JA, Bailey JK, Rehill BJ, Martinsen GD, Hart SC, Lindroth RL, Keim P, Whitham TG. 2004. Genetically based trait in a dominant tree affects ecosystem processes. *Ecology Letters* **7:** 127–134.

Schweitzer JA, Joseph K. Bailey JK, Hart SC, Whitham TG. 2005. Non-additive effects of mixing cottonwood genotypes on litter decomposition and nutrient dynamics. *Ecology* **86 (10)**: 2834–40.

Shantz AA, Burkepile DE 2014. Context-dependent effects of nutrient loading on the coral–algal mutualism. *Ecology* **95**: 1995-2005.

Shipley B. 2007. Comparative Plant Ecology as a Tool for Integrating Across Scales. *Annals of Botany* **99 (5):** 965–966.

Silfver T, Mikola J, Rousi M, Roininen H, Oksanen E. 2007. Leaf litter decomposition differs among genotypes in a local *Betula pendula* population. *Oecologia* **52 (4)**: 707–14.

Silfver T, Roininen H, Oksanen E, Rousi M. 2009. Genetic and environmental determinants of silver birch growth and herbivore resistance. *Forest Ecology and Management* **257 (10)**: 2145–49.

Silfver T, Paaso U, Rasehorn M, Rousi M, Mikola J. 2015. Genotype × herbivore effect on leaf litter decomposition in *Betula pendul*a saplings: ecological and evolutionary consequences and the role of secondary metabolites. *Plos one* **10(1)**: 1–15.

Supek F, Bošnjak M, Škunca N, Šmuc T. 2011. REVIGO summarizes and visualizes long lists of gene ontology terms. *PloS one* **6.7**: e21800.

Swift MJ, Andrén O, Brussard M, BRiones MM, Couteaux E, Ekschmitt A, Kjoller A, Loiseai P, Smith P. 1998. Global Change, Soil Biodiversity, and Nitrogen Cycling in Terrestrial Ecosystems: Three Case Studies. *Global Change Biology* **4 (7)**: 729.

Treseder KK, Vitousek PM. 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. *Ecology* **82 (4)**: 946–54.

Vaieretti MV, Pérez Harguindeguy N, Gurvich DE, Cingolani M, Cabido M. 2005. Decomposition dynamics and physico-chemical leaf quality of abundant species in a montane woodland in Central Argentina. *Plant and Soil* **278 (1–2)**: 223–34.

Vasseur F, Violle C, Enquist BJ, Granier C, Vile D. 2012. A common genetic basis to the origin of the leaf economics spectrum and metabolic scaling allometry. *Ecology Letters* **15**: 1149–1157

Vasseur F, Exposito-Alonso M, Ayala-Garay OJ, Wang G, Enquist BJ, Vile D, Violle C, Weige D. 2018a. Adaptive diversification of growth allometry in the plant *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences* **115(13)**: 3416-3421.

Vasseur F, Sartori K, Baron E, Fort F, Kazakou E, Segrestin J, Garnier E, Vile D, Violle C. 2018b. Climate as a driver of adaptive variations in ecological strategies in *Arabidopsis thaliana*. *Annals of Botany* **122 (6)**: 935-945.

Violle C, Enquist, BJ, McGill BJ, Jiang L, Albert, CH, Hulshof C, Jung V, Messier J. 2012. The return of the variance: intraspecific variability in community ecology. *Trends in Ecology and Evolution.* **27(4)**: 244-52.

Wardle DA, Bonner KI, Barker GM. 2002. Linkages between plant litter decomposition, litter quality. *Functional Ecology* **16**: 585–95.

Weigel D. 2012. Natural variation in *Arabidopsis*: from molecular genetics to ecological genomics. *Plant Physiology* **158 (1)**: 2-22.

Welti EAR, Joern A. 2015. Structure of trophic and mutualistic networks across broad environmental gradients. *Ecology and evolution* **5 (2)**: 326–334.

Whitham TG, Bailey JK, Schweitzer JA, Shuster SM, Bangert RK, LeRoy CJ, Lonsdorf E, Allan GJ, DiFazio SP, Potts BM, Fischer DG, Gehring CA, Lindroth RL, Marks J, Hart SC, Wimp GM, Wooley SC, 2006. A framework for community and ecosystem genetics: from genes to ecosystems. *Nature Reviews Genetics* **7**: 510e523.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M et al. 2004. The worldwide leaf economics spectrum. *Nature* **428 (6985):** 821–827.

Wright JP, Ames GM, Mitchell RM. 2016. The more things change, the more they stay the same? When is trait variability important for stability of ecosystem function in a changing environment *Philosophical Transactions of the Royal Society B: Biological Science* **371**: 20150272.

Wu S, Tohge T, Cuadros-Inostroza Á, Tong H, Tenenboim H, Kooke R, Méret M, Keurentjes JB, Nikoloski Z, Fernie AR et al. 2018. Mapping the *Arabidopsis* metabolic landscape by untargeted metabolomics at different environmental conditions. *Molecular Plant* **11(1)**: 118 - 134.

Zhu YH, Chen H, Fan J, Wang Y, Li Y, Chen J, Fan J, Yang S, Hu L, Mew TW et al. 2000. Genetic Diversity and Disease Control in Rice. *Nature* 406 **(6797)**: 718–722.

Zhou X, Carbonetto P, Stephens M. 2013. Polygenic modeling with bayesian sparse linear mixed models. *PLoS Genet*. **9**: e1003264.

Supplemental Figure S1. Variation in morpho-physiological traits measured on the 211 genotypes.

Supplemental Figure S2. Part of variance (%) explained by the main PCs.

Supplemental Figure S3. GO enrichment analysis for litter decomposability and metabolomic PC3.

Supplemental Methods S1. Description of the decomposition assay in the laboratory.

Supplemental Table S1. List of accessions with measured phenotypic traits.

Supplemental Table S2. Correlation of LMR with leaf traits, leaf-traits PCs and metabolomic PCs.

Supplemental Table S3. Sixty top contributors of metabolomic PC3.

Figure legends

Figure 1. Geographic origin of the 211 genotypes of *A. thaliana* used in this study.

Figure 2. Variation in litter mass remaining (LMR, %) across *A. thaliana* genotypes. For each accession, linked grey dots represent individual values ($n = 3$) and red dots represent mean genotypic value. Genotypes are ordered by increasing mean LMR values.

Figure 3. Relationships between morpho-physiological traits, metabolites and litter decomposability of *A. thaliana* (A) 'Morpho-physiological PCA' performed on seven traits: leaf area, specific leaf area (SLA), leaf nitrogen content (LNC), leaf lifespan (LLS), leaf tensile strength (LTS), leaf dry matter content (LDMC) and trichome density (Trichomes); as well as flowering time (FT). The two first PCs are represented. (**B**) 'Metabolomic PCA' performed on 2,132 metabolites extracted from Wu *et al*. (2018). Both PCAs were performed on the 91 genotypes with morpho-physiological and metabolomic data. For convenience, metabolites names were not represented. Morpho-physiological traits and metabolites were all scaled prior to PCAs. (**C**) Random forest analysis of the contribution of metabolomic and morpho-physiological PCs on LMR ($n = 91$). Only PCs that explained more than 5% of total phenotypic variation (*i.e*. PCs 1 to 4 for each PCA) were retained for further analyses (+/- SD). Stronger increase in MSE (mean square error) reflects more important contribution to LMR. (**D**) Results of multiple regressions to compare effect size and significance of

metabolomic and morpho-physiological PCs on LMR ($n = 91$). All PCs were scaled prior to analysis.

Figure 4. Correlations between litter decomposability and specific metabolites.
Metabolites classes were extracted from Wu *et al*. (2018) study. Correlations were estimated with Spearman's rank coefficient of correlation (ρ) between each individual metabolite and LMR. For convenience, amines and amino acids were treated together, as well as glucosides and sugars, and nucleoside, nucleotide and their derivatives ('Nucleoside' in the figure). Only the classes with at least three individual metabolites were analyzed. Blue points represent the coefficients of correlation between individual metabolites and LMR. Empty circles represent non-significant correlations ($P > 0.05$), and solid circles represent significant correlations ($P < 0.05$). Red dashed line represents zero axis (+/- SD).

Figure 5. Effect of climate of origin on litter decomposability. (**A**) 'Climatic PCA' performed on 19 variables extracted from the Worldclim database at the collection points of 211 *A. thaliana* genotypes. T: temperature (°C), P: precipitation (mm). (**B**) Results of chain regressions to compare effect size and significance of climate PCs on LMR ($n = 211$). All PCs were scaled prior to analysis (+/- SD).

Figure 6. Genetic analysis of litter decomposability. (**A**) Monogenic GWAS performed with EMMAX on LMR across 199 genotypes with genetic markers (214K SNPs). Dot colors represent chromosomes. Red lines represent significance thresholds (Bonferroni correction at $\alpha = 0.05$ and $\alpha = 0.1$ for solid and dashed lines, respectively). (**B**) Gene Ontology (GO) enrichment analysis for LMR, performed with REVIGO online tool. Box size proportional to significance ($\log_{10}(P\text{-value})$) of the GO category. Only the cellular component class is represented. (**C**) GO enrichment analysis for metabolomic PC3, performed with REVIGO online tool. Box size proportional to significance ($\log_{10}(P\text{-value})$). Only the cellular component class is represented. (**D**) Correlation between SNP effect on metabolomic PC3 and SNP effect on LMR. SNP effect estimated with BSLMM. r is Pearson's coefficient of correlation. Dashed line represents linear regression.
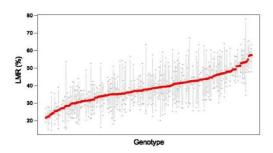
Figure 1

Figure 2
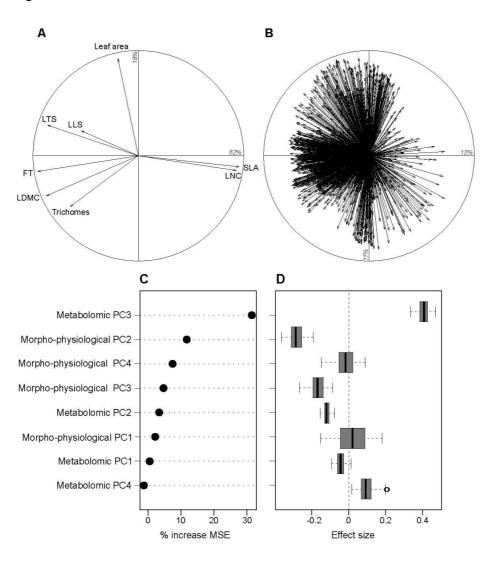
Figure 3

Figure 4

Figure 5

Figure 6