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**Metabarcoding and ecological interaction networks for  
selecting candidate biological control agents**

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5 Mélodie Ollivier<sup>1</sup>, Vincent Lesieur<sup>1,2</sup>, Johannes Tavoillot<sup>3</sup>, Fanny Bénétière<sup>1</sup>, Marie-Stéphane  
6 Tixier<sup>1</sup>, Jean-François Martin<sup>1</sup>

7 <sup>1</sup> CBGP, Montpellier SupAgro, INRAE, CIRAD, IRD, Univ Montpellier, Montpellier,  
8 France

9 <sup>2</sup> CSIRO Health and Biosecurity, European Laboratory, Montferrier sur Lez, 34980, France

10 <sup>3</sup> CBGP, IRD, CIRAD, INRAE, Montpellier SupAgro, Univ Montpellier, Montpellier,  
11 France

## 12 Abstract

- 13 1. Classical biological control can be used to decrease the density of invasive species to below  
14 an acceptable ecological and economic threshold. Natural enemies specific to the invasive  
15 species are selected from its native range and released into the invaded range. This approach  
16 has drawbacks, despite the performance of specificity tests to ensure its safety, because the  
17 fundamental host range defined under controlled conditions does not represent the actual  
18 host range *in natura*, and these tests omit indirect interactions within community.
- 19 2. We focus on *Sonchus oleraceus* (Asteraceae), a weed species originating from Western  
20 Palearctic that is invasive worldwide and notably in Australia. We explore how analyses of  
21 interaction network within its native range can be used to 1) inventory herbivores associated  
22 to the target plant, 2) characterize their ecological host ranges, and 3) guide the selection of  
23 candidate biocontrol agents considering interactions with species from higher trophic  
24 levels. Arthropods were collected from plant community sympatric to *S. oleraceus*, in three  
25 bioclimatic regions, and interactions were inferred by a combination of molecular and  
26 morphological approaches.
- 27 3. The networks reconstructed were structured in several trophic levels from basal species  
28 (diversified plant community), to intermediate and top species (herbivorous arthropods and  
29 their natural enemies). The subnetwork centered on *S. oleraceus* related interactions  
30 contained 116 taxa and 213 interactions. We identified 47 herbivores feeding on *S.*  
31 *oleraceus*, 15 of which were specific to the target species (*i.e.* Generality value equal to 1).  
32 Some discrepancies with respect to published findings or conventional specificity tests  
33 suggested possible insufficient sampling effort for the recording of interactions or the

34 existence of cryptic species. Among potential candidate agents, 6 exhibited interactions  
35 with natural enemies.

36 4. *Synthesis and applications*: Adopting a network approach as prerequisite step of the CBC  
37 program can provide a rapid screening of potential agents to be tested in priority. Once  
38 ecological host range defined, we suggest that priority should be given to agent predated by  
39 a minimum species, and, when they exist, to an agent that possesses enemies from the most  
40 distant taxonomical group from those occurring in the range of introduction.

41 **Key words**: High-throughput sequencing, host range, food web, weed biocontrol, Common  
42 Sowthistle

43

## 44 Introduction

45 Biological invasions are currently threatening biodiversity to an unprecedented extent (Bellard et  
46 al., 2016; Seebens et al., 2015; Vitousek et al., 1997). When invasive species disrupt the  
47 ecological or economic balance, action is required to control their negative impact. Chemical  
48 control methods are widely used in such situations, but classical biological control (CBC)  
49 constitutes a possible alternative. CBC involves the release of natural enemies, specific to the  
50 target organism and originating from its native range, to keep the density of the invasive species  
51 below an economically and ecologically acceptable threshold (Keane and Crawley, 2002;  
52 McFadyen, 1998; Van Driesche et al., 2010). CBC is considered more sustainable than chemical  
53 control (Peterson et al., 2020), although the introduction of biocontrol agents (BCA) into a new  
54 territory may itself represents a risk for the recipient communities (Barratt et al., 2018; Hinz et  
55 al., 2019; Suckling and Sforza, 2014). Once introduced, the BCA may affect non-target species,  
56 especially if it lacks specificity (Müller-Schärer and Schaffner, 2008). Assessing the host range of  
57 a candidate BCA is, thus, crucial, to anticipate such risks. Most of the host-specificity tests  
58 performed to assess this risk are conducted under standardized conditions, through choice/no-  
59 choice experiments over a range of targets selected according to the centrifugal phylogeny  
60 approach (Briese, 2005; Wapshere et al., 1989).

61 Recent reviews recognized the success of such experiments for limiting the undesirable  
62 unintentional effects of the CBC of weeds (Hinz et al., 2019, 2020). However, the cumbersome  
63 nature of these tests reduces the range of species that can be screened. The candidate BCA are  
64 selected through preliminary field monitoring that may miss a species of interest. Furthermore, as  
65 the fundamental host range of a species (defined under controlled conditions) is thought to be  
66 broader than the host range actually observed in the field (known as the realized host range;

67 Louda et al., 2003; Schoonhoven et al., 1998; Sheppard and Harwood, 2005), these tests tend to  
68 overestimate the risk and lead to the rejection of candidate BCA based on interactions that would  
69 not occur in the field (false positives) (e.g. Groenteman et al. 2011). Most CBC programs use  
70 specificity tests under controlled conditions as proxies for field conditions due to the complexity  
71 of trophic interaction assessments in the field but this leaves room for improvement.

72 The characterization of ecological interactions among communities of plants and arthropods *in*  
73 *natura* is challenging, as it traditionally requires direct observations, the rearing of specimens and  
74 considerable taxonomic expertise, rendering the process impractical for large-scale studies.

75 Recent advances in molecular approaches, such as the combination of DNA metabarcoding on  
76 gut content or feces and high-throughput next-generation sequencing (NGS), have opened up new  
77 opportunities to track the host range of arthropods *in natura* with both a high taxonomic  
78 resolution and high sensitivity (Derocles et al., 2018; Frei et al., 2019; Wirta et al., 2014; Zhu et  
79 al., 2019). Even interactions that are very difficult to observe, such as host-parasitoid  
80 associations, can be detected by such methods (Garipey et al., 2014; Hrček and Godfray, 2015).

81 This approach can be used to reconstruct networks of trophic interactions directly from studies in  
82 the field, and provides an analytical framework particularly relevant to studies of complex  
83 species assemblages. Network ecology do not only depicts species interactions, but provides  
84 elements for the understanding of recurrent patterns of antagonistic interactions between plants  
85 and herbivores, such as specialization or compartmentation (Lewinsohn et al., 2006; Thébault and  
86 Fontaine, 2010). In CBC against invasive weeds, analyses of ecological networks have been used  
87 to assess the extent to which a BCA fits into a recipient community. Such methods provided a  
88 way to quantify the direct impact of biological control on non-target plants (Memmott, 2000),  
89 and its indirect impact on other species at higher trophic levels (Carvalho et al., 2008; Louda et

90 al., 1997; Pearson and Callaway, 2003). Such studies have highlighted the usefulness of network  
91 ecology for evaluating the impact of BCA after their introduction (Memmott, 2009; Willis and  
92 Memmott, 2005), but interaction network analysis can also be used for the upstream assessment  
93 of potential candidate BCA, in a more systematic process (Ollivier et al., 2020). Adopting a  
94 network approach as prerequisite step of the CBC program, can provide a rapid screening of the  
95 ecological host range of potential agents to be tested in priority. This can also inform about  
96 species functional properties through the position and connexions the species have in the  
97 network, independently of its taxonomic assignation, which would confer a strong predictive  
98 power of the interactions possibly occurring in a novel bioclimatic region (Todd et al., 2020).  
99 Indeed, the choice of BCA should also take into account indirect effects on the recipient  
100 community due to interactions with higher trophic levels in the network, *i.e.* natural enemies  
101 (Hinz et al., 2019; Memmott, 2000). If comparable enemies than those identified in the native  
102 range are present in the range of introduction, new interactions might be created with BCA,  
103 resulting in disturbances in the ecological network through indirect interactions, *e.g.* apparent  
104 competition (Carvalho et al., 2008; López-Núñez et al., 2017).

105 The objective of this study was to determine how the analysis of interaction networks could be  
106 used to support the selection of candidate BCA for the common sowthistle, *Sonchus oleraceus* L.  
107 (Asteraceae). This plant is native to Western Europe and Northern Africa (Boulos, 1974;  
108 Hutchinson et al., 1984) and is the most widely naturalized terrestrial plant worldwide (Pyšek et  
109 al., 2017). In Australia, it has become a weed of major concern in cropping systems (Llewellyn et  
110 al., 2016; Widderick et al., 2010). Aside the development of resistance to multiple herbicides  
111 (Adkins et al., 1997; Jalaludin et al., 2018; Meulen et al., 2016), the control of this weed is  
112 complex as it is extremely prolific and seeds can germinate all year round when sufficient



113 rainfalls occurs. *Sonchus oleraceus* rapidly dominates crops, reducing yield and contaminating  
114 harvested grain (Llewellyn et al., 2016). A CBC program was therefore initiated in 2017, to  
115 identify candidate BCA. In this context, an analysis of ecological networks, based on direct field  
116 observations and high-throughput DNA metabarcoding, was performed. Our objectives were to  
117 1) establish an inventory of arthropods feeding on *S. oleraceus*, and assess the contribution of the  
118 approach relatively to classical procedures, 2) delineate the ecological host range for herbivores  
119 feeding on *S. oleraceus* and point out candidate BCA, and 3) identify the trophic interactions of  
120 the candidate BCA with natural enemies, and consider their implications for the CBC program.

121

## 122 Materials and Methods

### 123 Sampling design

124 We maximized the species diversity and associated interactions, through a maximum variation  
125 design with three bioclimatic regions in France (semi-oceanic, Mediterranean and continental  
126 climates) (Ceglar et al., 2019) and three successive sampling dates (April, May and June 2018).  
127 Sampling was carried out from 10 A.M. to 4 P.M., by varying climatic conditions (wet, cloudy to  
128 sunny weathers and temperatures ranged between 10°C and 29°C). These variations did not affect  
129 our ability to capture arthropods. For each bioclimatic region and date, we employed an  
130 opportunistic sampling strategy to collect plants from several ruderal and agricultural sites,  
131 covering the diversity of habitats (open and disturbed) colonized by *S. oleraceus* (Supplementary  
132 Table 1). At each site, on each date, we sampled three quadrats (1 m<sup>2</sup>) along a 20 m linear  
133 transect. Quadrats were placed to contain at least one *S. oleraceus* plant. Within each quadrat,  
134 arthropods were collected from plants with a forceps or brush, and stored individually in sterile 2  
135 ml Eppendorf tubes filled with a protective buffer solution. This solution is used to prevent  
136 oxidation of polyphenols and polyamines (PCR inhibitors) (see Cruaud et al. (2018) for more  
137 details). This procedure was repeated for each plant of every plant species present in the quadrat  
138 over a period of one hour, to standardize the sampling effort. This period was deemed adapted to  
139 represent the biodiversity of the sampled unit, and to allow vagrant insects, potentially disturbed  
140 by our arrival, to settle back on their resource plant before sampling. We collected individual  
141 specimens except for colonies of aphids, thrips, and egg masses, for which at least five specimens  
142 were required to obtain sufficient DNA for analysis. We did not consider pollinators or the soil  
143 fauna in this study. Following the collection of each specimen, tools were thoroughly cleaned by  
144 successive immersions in 2.5 % bleach solution, water and 96% ethanol, to prevent cross-

145 contamination. At the end of the one-hour insect sampling period, all the plants within the  
146 quadrat were collected individually (by cutting the stem at the soil surface), for further dissection.  
147 Back in the laboratory, the plants were identified morphologically, and their organs (stems,  
148 leaves, flowers) were dissected to collect endophagous arthropods, which were transferred into  
149 tubes as described for the arthropods collected in the field. For each arthropod specimen  
150 collected, we identified the plant species from which arthropods were sampled, and recorded the  
151 specimen stage and condition (degraded, parasitized), and putative identification (at least  
152 taxonomic group, with identification to species level if straightforward). All arthropod samples  
153 were frozen at -20°C until DNA analysis. Thus, while plants were identified morphologically,  
154 arthropods were identified via molecular technologies. Each plant was transferred to a paper bag  
155 and oven-dried at 70°C for 72 h, for the determination of aboveground dry biomass (g) as an  
156 estimate of plant abundance per quadrat. Arthropod abundances were determined based on the  
157 number of individuals collected per quadrat for each taxon. Sampling was performed for 57  
158 quadrats, over the three sampling dates.

### 159 [High-throughput DNA metabarcoding](#)

160 We characterized the interaction network by directly observing plant-arthropod interactions  
161 (recording only interactions for which an observation of feeding was verified); while arthropod-  
162 arthropod interactions were revealed by molecular analysis. We first isolated total DNA from  
163 each arthropod individual (Cruaud et al., 2018). As presented in Supplementary Figure S1, we  
164 then performed metabarcoding on each arthropod sample, with a two-step DNA amplification  
165 and high-throughput sequencing method adapted from the procedure described by Galan et al.  
166 (2017). We sequenced three short COI fragments, with primer combinations and PCR protocols  
167 developed elsewhere (HCO forward: Leray et al. 2013, HCO reverse: Folmer et al. 1994, LEP F.

168 and R.: Brandon-Mong et al. 2015, HEX F. and R.: Marquina et al. 2019), to overcome the  
169 problem of the lack of primer universality among arthropods. Error-proof indices for individual  
170 sample identification were developed with the high-throughput sequencing process described by  
171 Martin (2019). The libraries were sequenced with Illumina technology, using a Miseq 2x250 run  
172 for date 1 (April), and one lane of Hiseq 3000 each for dates 2 (May) and 3 (June).

173 The markers for each sample were demultiplexed with CutAdapt v2.3, and all paired-end reads  
174 were filtered for minimal length (280 bp), corrected for sequencing errors, and pairs of  
175 overlapping reads were merged with the Dada2 v1.12 R package (Callahan et al., 2016). A matrix  
176 was thus obtained, containing samples as variables and amplicon variant sequences (ASVS) as  
177 observations. A variant is a set of identical corrected and merged paired-end reads. We used  
178 Qiime2 (Bolyen et al., 2018) with a 2% divergence threshold, to merge ASVS, to decrease their  
179 number without the loss of taxonomic information. The summed number of reads for each  
180 merged variant for a given arthropod sample was reported as the intersection of samples and  
181 ASVS.

182 Each ASVS was assigned, by BLAST, to a barcoding reference database of cytochrome oxidase  
183 subunit I (COI) nucleotide sequences (658 bp) compiled from three different sources and curated  
184 by expert analysis. These reference barcodes were retrieved from BOLDSYSTEM  
185 (Ratnasingham and Hebert, 2007), the CBGP - Continental Arthropod collection (Centre de  
186 Biologie pour la Gestion des Population, 2019) and a local database specifically designed for this  
187 study. Our database contained barcodes of the most frequently encountered species during this  
188 sampling campaign (extra-specimens collected) and field surveys (2017-2020) conducted through  
189 Europe and North Africa for the search of *S. oleraceus* natural enemies (see below). In total,  
190 these three sources compiled 1 699 995 sequences from 119 299 species available for ASVS

191 assignment. We retained successful assignments to the ranks of species, genus and family, but not  
192 those to higher taxonomic levels, because arthropod biology is too variable at higher taxonomic  
193 ranks to be informative for our purpose. The assignments obtained for each marker were grouped  
194 together in a single table and the numbers of reads were summed by assigned taxon. The resulting  
195 file was therefore an interaction matrix in BIOM file format, in which the assigned taxa replaced  
196 ASVS. The matrix was curated and manually transformed to obtain an adjacency matrix (in  
197 which the observations are sources and the variables are consumers) usable for further network  
198 analyses. For each pair of consumer/prey species, occurrence frequencies of interaction were  
199 calculated (Supplementary Text 1 and Figure S2).

#### 200 Assessment of sampling robustness and global network description

201 We first evaluated the completeness of sampling over the entire sampling campaign, and  
202 generated taxon accumulation curves (the 57 quadrats were added in a random order, with 1,000  
203 permutations) for plants and arthropods, using the *specaccum* function of the R package *vegan*  
204 (Oksanen *et al.*, 2019). We estimated the extrapolated taxonomic richness by calculating the  
205 Chao 1 index (Chao, 1984) with the *specpool* function. Likewise, the robustness of sampling for  
206 the characterization of interactions was assessed by generating accumulation curves for pairwise  
207 interactions. We first generated an accumulation curve including all the types of direct  
208 interactions (*e.g.* plant-herbivores, herbivores-natural enemies, etc.) present in the meta-network  
209 (*i.e.* pooling interactions from all sites). The 57 quadrats were added in a random order, with  
210 1000 permutations. We finally generated a curve focusing on interactions involving *S. oleraceus*  
211 as a source, to evaluate the performance of the sampling design for addressing our objective of  
212 establishing an inventory of the arthropods feeding on *S. oleraceus*, corresponding to candidate

213 BCA. For both curves, we estimated the extrapolated interaction richness with the Chao 1 index  
214 (Chao 1984), using the *specpool* function.

215 Prior to interaction analyses, a global description of the meta-network (pooling interactions data  
216 from all sites) and subnetwork (centred on *S. oleraceus* related interactions) was performed.

217 Several metrics were calculated: the number of links (L), the number of nodes (S) (connected and  
218 isolated), connectance (C) and link density (LD) (Bersier et al., 2002; Warren, 1994).

219 Connectance is the proportion of the possible trophic links actually realized; here cannibalism is  
220 not permitted, so  $C = L/S(S-1)$ . Link density is the mean number of links per taxon, calculated as  
221  $LD = L/S$ . We also characterized the taxon assemblage by determining taxonomic richness (*i.e.*  
222 number of taxa) for each trophic level (plants, herbivores and natural enemies).

### 223 Selection of candidate biocontrol agents

224 The selection of candidate BCA was decided according two criteria: a restricted ecological host  
225 range and limited interactions with natural enemies. Thus, based on the interactions retrieved  
226 from the meta-network, we selected a subnetwork considering only the arthropods having *S.*  
227 *oleraceus* as a source plant, as well as all their complementary plant resources. We also included  
228 natural enemies associated with these herbivores (*i.e.* parasitoids and predators). We assessed and  
229 visualized the specificity of these herbivores, by plotting interactions between herbivores  
230 encountered on *S. oleraceus* and all their complementary resource plants as a grid matrix, in  
231 which plants were ordered by their degree of phylogenetic relatedness to *S. oleraceus*, as defined  
232 by the current classification of angiosperms (Chase et al., 2016). Arthropods were ordered by the  
233 increasing generality values (*i.e.* the number of resources per taxon) characterizing ecological  
234 host range. To assess and visualize the dependence of natural enemies on these herbivores, we  
235 constructed a second level grid matrix in connexion with the previous, and calculated arthropod

236 vulnerability values (*i.e.* the number of consumer per taxon). Multipartite network and grid  
237 matrices were constructed with *igraph* R package.

238 Assessing the contribution of the approach for the biocontrol program

239 To discuss the contribution of the method herein proposed, we used, as a point of reference, a  
240 survey performed following classical procedures (sampling, rearing and identification of  
241 specimens exclusively collected from *S. oleraceus*) in the frame of this CBC program (Lesieur et  
242 al., in prep). However, we acknowledge that this classical survey covered a longer period of  
243 sampling (2017-2020) and a much larger geographical area was prospected (10 countries through  
244 Europe and North Africa).

## 245 Results

246 Summary of the molecular results

247 In total, 2,834 arthropod specimens were collected and analyzed by metabarcoding, to reconstruct  
248 the interaction network at a global scale. We obtained DNA sequences and taxonomic  
249 assignments for 1,803 of the 2,834 arthropods initially collected (63.6 %). This proportion of  
250 exploitable information reached 71% (2,011 specimens) after manual validation of the matrix.  
251 The molecular analysis provided a total of 107,483,410 reads, 19.2% of which were retained after  
252 screening with quality filters; we obtained a final dataset of 2,014 COI variant sequences  
253 (Supplementary Table 2). Before, manual validation, we observed that a large proportion of the  
254 diversity (33% of the families and 40% of the species) was recovered by the use of all markers,  
255 the rest being recovered by a combination of two markers, or specifically found with only one  
256 marker (Supplementary Figure S3). LEP increased identification rates by 20% for families and  
257 25% for total species, consistent with its widespread use in the research community (Brandon-  
258 Mong et al., 2015). The other two markers also provided original information, albeit to a lesser

259 extent, at least as far as the number of taxa recovered was concerned, as 15% of the families and  
260 16% of the species would not have been recovered with LEP alone. After data validation, 269  
261 taxa were identified for arthropods, with 84% identified to species level (17 orders, 90 families  
262 and 189 genera). While plant taxonomic diversity (relying on morphological identifications)  
263 accounted for 132 taxa, 80% of which were classified to species level (25 orders, 29 families and  
264 87 genera) (Supplementary figure S4).

### 265 **Sampling robustness**

266 The accumulation curve of plants seemed to approach an asymptote, but this was not the case for  
267 arthropods (Figure 1). The Chao 1 index indicated an extrapolated taxonomic richness value for  
268 plants of 164 taxa ( $\pm 12$ ), with 132 taxa actually sampled. By contrast, for arthropods, the  
269 extrapolated taxonomic richness value was 442 taxa ( $\pm 39$ ), but only 269 taxa were actually  
270 sampled. Sampling robustness was high over the entire sampling scheme for plants but sampling  
271 efficiency was lower for arthropods. Likewise, we assessed the completeness of pairwise  
272 interactions detected over the whole network. We observed a linear increase associated with a  
273 Chao1 index of 1245 ( $\pm 183$ ) expected interactions, where 350 links were actually reconstructed  
274 (Figure 1). However, this is less of an issue for interactions involving *S. oleraceus*, the focus of  
275 the analysis for which this sampling was designed. The accumulation curve in question tended  
276 towards an asymptote, with a Chao 1 index of 63 ( $\pm 10$ ) expected interactions and 47 interactions  
277 sampled. Overall, these results suggest that the sampling effort was adequate for the  
278 reconstruction of a unique interaction network maximizing of the proportion of links observed  
279 (Jordano, 2016).



## 280 [Meta-network and subnetwork analyses](#)

281 As presented in Table 1, the complete interaction network (meta-network) consisted of 401  
282 nodes, 241 of which were connected to another node (60%), resulting in 350 links  
283 (Supplementary Figure S5). Linkage density and connectance calculated were 1.45 and 0.006,  
284 respectively. The meta-network included 60 plants in interaction (46% of the plants collected),  
285 136 herbivores in interaction (74% of the herbivores collected), 35 natural enemies in interaction  
286 (49% of the natural enemies collected) comprising 19 parasitoid and 16 predator taxa, and 10  
287 omnivores (feeding at more than one trophic level). The sub-network consisted of 116 nodes and  
288 213 links, and resulting linkage density and connectance were 1.84 and 0.008, respectively  
289 (Figure 2). A more detailed description of taxon assemblage composing *S. oleraceus* subnetwork  
290 is provided in the following section.

## 291 [Identifying candidate biocontrol agents: considering host range and regulation by enemies](#)

292 Analysing *S. oleraceus* subnetwork, we found 47 herbivorous taxa feeding on the target,  
293 including 37 taxa identified to species level. They belonged to five different orders, *i.e.*  
294 Hemiptera (45%), Diptera (25%), Coleoptera (19%), Lepidoptera (0.06%) and Hymenoptera  
295 (0.04%), and were distributed in nine different trophic guilds, with the flower bud sucking-  
296 piercing guild being the most represented (23%) while the less represented guild corresponded to  
297 the chewing guild (2%) (Table 2). Fifteen taxa were collected exclusively from *S. oleraceus*, and  
298 another two taxa were collected from *S. oleraceus* and *Sonchus asper* (Figure 3). These taxa are  
299 potential BCA (host range apparently restricted to the genus *Sonchus*, subtribe Sonchinae). Six  
300 additional species were detected only on members of the tribe Chicorieae (*Aphis craccivora*  
301 Koch, *Ophiomyia cunctata* Hendel, *Phytomyza lateralis* Fallén, *Campiglosa producta* Loew, *L.*  
302 *punctiventris* and *T. formosa*). We identified 38 other plant species as complementary resource

303 plants for the herbivore species collected from *S. oleraceus*. The generality of these herbivore  
304 species ranged from 1 to 18, with *Philaenus spumarius* L. the most polyphagous of the 47  
305 herbivores species found on *S. oleraceus* (Figure 3).

306 The analysis of the subnetwork (Figure 2) also indicated that the herbivores collected on *S.*  
307 *oleraceus* were a resource for diverse natural enemies. In particular, 19 of the 47 herbivorous taxa  
308 collected were attacked by several species of parasitoid (12 species from the family Braconidae, 1  
309 from Figitidae, and 1 from Ichneumonidae) and predators (6 Arachnida species, 1 from  
310 Cantharidae, 2 from Coccinellidae, 3 from Syrphidae and 1 from Orthoptera). Moreover, among  
311 the 17 arthropods identified as candidate BCA for their restricted ecological host range, we  
312 detected interactions with natural enemies for six of them, one exhibiting interactions with 8 taxa  
313 from higher trophic levels (Figure 3).

314 Eventually, molecular analyses revealed particular patterns of omnivory involving several species  
315 from Heteroptera. We distinguished between intermediate omnivores (species feeding on both  
316 plants and herbivores, such as members of the Tephritidae and Aphididae), and top omnivores  
317 (species feeding on herbivores and natural enemies, such as members of the Syrphidae). The list  
318 of the taxa included in the subnetwork and of all the trophic interactions (*i.e.* the edge list) used to  
319 generate Figure 3 are provided in Supplementary Tables 3 and 4, respectively.

320 Assessing the contribution of the approach for the biocontrol program

321 The analysis of trophic interactions identified 47 taxa feeding on *S. oleraceus*, 37 of which were  
322 identified to species level. Nineteen of these 37 species had already been sampled in classical  
323 field surveys, the other 18 species being newly reported as herbivores of *S. oleraceus* in this CBC  
324 program (Table 2).

## 325 Discussion

### 326 The combination of observation and molecular data performs well for the characterization 327 of interactions

328 The metabarcoding approach used made it possible to target a broad diversity of taxa (90  
329 arthropod families, with identification to species level of 84% of the variants), as expected with  
330 the use of multiple markers (Alberdi et al., 2018; Creedy et al., 2019; Marquina et al., 2019). The  
331 combination of taxonomic assignments with subsequent observational data and information  
332 available in the literature was essential: 1) to validate the trophic links (predation and parasitism)  
333 and 2) to complement the identification in cases of failed amplification or taxonomic assignment,  
334 as advocated in other contexts (Derocles et al., 2018; Wirta et al., 2014). In the meta-network,  
335 60% of the taxa interacted, suggesting that our methods performed very well for the  
336 reconstruction of interactions. More specifically, interaction detection rates obtained for plant-  
337 herbivores and herbivores-natural showed higher values than those usually reported in  
338 comparable contexts (Braukmann et al., 2017; Clare, 2014; Erickson et al., 2017; García-Robledo  
339 et al., 2013; Roslin and Majaneva, 2016). The high rate of interaction reported here for  
340 herbivorous arthropods can be explained by our decision to focus on intensive plant dissection  
341 and morphological determination. Retaining feeding interactions only after verification reduced  
342 the risk of false positives, over-estimating species interactions, related to the use of co-occurrence  
343 data (*i.e.* tourist insects on plants rather than actual trophic links) (Zhu et al., 2019). For  
344 arthropods, the rather low rate of natural enemies positive for preys can be multifactorial; *e.g.*  
345 mismatch between the primer pairs used and the prey species, low sequencing depth given the  
346 DNA yield ratio between consumer and prey, and degradation of DNA from consumed preys

347 (Hosseini et al., 2008; Macías-Hernández et al., 2018; Sheppard and Harwood, 2005; Sheppard et  
348 al., 2004).

### 349 [A complementary inventory of herbivores feeding on \*S. oleraceus\*](#)

350 The 47 taxa collected from *S. oleraceus*, covered a wide range of trophic guilds. This evidenced  
351 that the method herein employed did not bias the selection towards a particular trophic guild but  
352 allows the detection of herbivores exhibited diversified feeding habits. The selection of one or  
353 several BCA from these trophic guilds could offer a good complementarity of actions (Buccellato  
354 et al., 2019). Although the guild of flower head sucker-piercer was the richest, further dedicated  
355 experiments would be necessary to assess whether those candidate agents actually provide the  
356 best regulation action of *S. oleraceus* (Morin et al., 2009).

357 Several elements indicated that our approach is a good complement of the classical survey. For  
358 example, we provided a more detailed description of pollen chewers (only *Brassicogethes aeneus*  
359 (Fabricius, 1775) was recorded in the classical survey). For Diptera, the Tephritidae flies,  
360 *Tephritis cometa* Loew and *T. vespertina* Loew, are newly recorded. A phylogeny of this taxa  
361 based on the CO1 barcode showed that those species are closely related to *T. formosa* Loew  
362 (Smit et al., 2013), and we cannot, therefore, rule out possible molecular misidentification due to  
363 the short CO1 barcode used or host race differentiation, as frequently observed in this group  
364 (Diegisser et al., 2006). During the classical survey, despite intensive collections, the Tephritidae  
365 species *Campiglossa producta* has been sampled only on *S. oleraceus* from the Canary Islands.  
366 The intensive sampling efforts put in the present study on *S. oleraceus* led to the collection of  
367 rare *C. producta* specimens (only 15 specimens from two sites) in the continental bioclimatic  
368 region in France. This highlights that, despite a reduced area prospected and a limited time frame,  
369 some rare species were sampled and their ecological host range described. We acknowledge we

370 missed some species occurring later in the season or out of the sampled area. For example,  
371 *Cystiphora sonchi* Vallot (Diptera: Cecidomyiidae) was collected in the classical surveys and  
372 passed specificity tests (Lesieur et al., 2020), but was not sampled from *S. oleraceus* in the three  
373 bioclimatic regions from April to June. This lack of detection in our sampling campaign was  
374 expected, as rates of infestation with this species peak in summer (Rizzo and Massa, 1998).  
375 Hence, this study should be regarded as a complement of usual procedures. However, with the  
376 rapid development of molecular technologies and associated drop in price (Kennedy et al., 2020),  
377 we believe that this approach will be soon applicable at larger sampling scales.

## 378 Selection of BCA

### 379 First criterion: a restricted ecological host range

380 Based on the present results, 15 of the 47 herbivores feeding on *S. oleraceus* seemed to have an  
381 ecological host range restricted to *S. oleraceus*, and another two taxa appeared to be restricted to  
382 the genus *Sonchus*. All these taxa recovered from the ecological network are, thus, of particular  
383 interest as candidate BCA. However, contradictions were observed between the ecological host  
384 range described by network analysis and published findings or specificity test results (Lesieur et  
385 al., in prep). These discrepancies may be due to insufficient sampling for the recording of species  
386 interactions (as shown by accumulation curve on total interactions) or to the presence of cryptic  
387 host races or cryptic species that have yet to be deciphered. Additional studies will be required to  
388 characterize the ecological host ranges of these species further. In particular, two species,  
389 *Liriomyza sonchi* Hendel and *Ensina sonchi* L., were found associated with *S. oleraceus* and *S.*  
390 *asper* and were, therefore, considered to be candidate BCA because these plants are both invasive  
391 weeds in Australia (Cullen et al., 2012). However, a wider range of food resources has been  
392 reported in literature for these two species (Table 2). Conversely, the promising galling insect *T.*

393 *formosa* passed specificity tests during the traditional phase of the CBC program. It was found to  
394 be restricted to the genus *Sonchus*, contrary to the results reported here, as we found *T. formosa*  
395 on *Crepis vesicaria* L. (belonging to Cripidinae, the same tribe but a different subtribe to *S.*  
396 *oleraceus*). This plant was not intended to be tested as a potential food plant for *T. formosa*, and  
397 these results therefore highlight the complementarity of the ecological network approach for  
398 clarifying herbivore host range.

399 Moreover, in the interaction network, *C. producta* was identified on three different plant species  
400 from the Chicorieae tribe. This species, found only on *S. oleraceus* in the Canary Islands during  
401 the classical surveys, was considered a promising BCA for testing. The results presented here  
402 indicate that its host range would not be compatible with its use as a BCA, potentially leading to  
403 its exclusion from the list of candidate BCA. This example shows how the network developed  
404 here is complementary to classical procedures, making it possible to narrow down the list of  
405 candidate BCA to be tested.

406 The same applies to *Cheilisia latifrons* Zetterstedt, a species collected in the classical survey. In  
407 our study, we did not sample this species on *S. oleraceus*, but the meta-network indicated it was  
408 collected from *S. asper* and *Picris echioides* (L.), revealing its oligophagous dietary behavior.  
409 However, little is known about the biology and the host plants of *C. latifrons* (Schmid and  
410 Grossmann, 1996) and its taxonomy seems to be unsolved, calling into question the existence of a  
411 species complex defined on the basis of host plant use (Speight, 2014). We also observed  
412 discrepancies for specimens from Cynipidae that appeared to be generalist herbivores in the  
413 interaction network (associated with both *S. oleraceus* and *Carduus pycnocephalus* Spreng.),  
414 whereas subsequent analysis of the variants assigned to Cynipidae indicated a genetic structure  
415 more consistent with multiple cryptic species potentially specializing on the host plants from

416 which they were collected. One species from Cynipidae is a known stem galler of *S. oleraceus*:  
417 *Aulacidea follioti* Barbotin (Bladmineerders Online database, 2020). However, this species is not  
418 yet present in any of the barcoding databases used here and could therefore only be assigned to  
419 family level. Further prospections to collect other Cynipidae specimens and rear them to  
420 adulthood would be required to confirm this identification.

#### 421 [Second criterion: limited interactions with natural enemies](#)

422 By using metabarcoding to reconstruct interactions between arthropods, we were able to detect a  
423 wide range of parasitoids from their herbivore hosts, and some predators. We detected  
424 omnivorous dietary behavior in several groups from Heteroptera. Opportunistic predation through  
425 carnivory is common in Lygaeidae (Burdfield-Steel and Shuker, 2014) to supplement the low  
426 levels of protein supplied by plants. Carnivory has also been reported in Miridae (Wheeler, 2001)  
427 and sometimes leads to intraguild predation interactions. We found that both Syrphidae (Diptera)  
428 and Miridae (Heteroptera) fed on aphid species, but we also revealed that mirids could prey upon  
429 syrphids, as already demonstrated in arena experiments (Fréchette et al., 2006). Members of the  
430 Lygaeidae and Miridae were also found to be able to access and feed on larval stages of several  
431 Tephritidae species whilst inside the flower heads of *S. oleraceus*. This interaction does not seem  
432 to have been observed before and provides insight useful not only for the CBC program against *S.*  
433 *oleraceus*, but also with direct implications for other biological control programs, particularly  
434 those involving the conservation biological control of insect pests.

435 More specifically, among candidate BCA exhibiting a restricted ecological host range, some were  
436 associated to an important diversity of natural enemies, and should be considered of lower  
437 priority for testing (*i.e.* *Ensina sonchi*). We suggest that priority should be given to agent  
438 predated by a minimum parasitoid and predator species, and, when they exist, to an agent that

439 possesses enemies from the most distant taxonomical group from those occurring in the range of  
440 introduction (Ollivier et al., 2020). It has been shown that newly created interactions between  
441 hosts and parasitoids in the introduced range are predictable based on the realised interactions in  
442 native range (Paynter et al., 2017; Veldtman et al., 2011). Further steps in this program would  
443 consist in investigating the diversity of natural enemies occurring in the range of invasion to  
444 anticipate new potential interactions and refine BCA choice.

## 445 Conclusion

446 We demonstrate here the potential of network ecology for characterizing candidate BCA and  
447 their ecological interactions in the field. This characterization clearly benefited from the use of  
448 complementary approaches (morphological and molecular analyses) to identify plant/arthropod  
449 and arthropod/arthropod interactions and provided a solid framework for the establishment of an  
450 inventory of herbivores feeding on the target weed, their realized host range and interactions with  
451 natural enemies. Avenues for further investigation have been identified and in-depth studies are  
452 now required. The strength of this approach also lies in its capacity to screen field host ranges for  
453 multiple herbivore species simultaneously, without the need for as many tests as species. This  
454 potential to narrow down the list of candidate BCA for testing should help to save both time and  
455 money. Finally, in addition to the potential value of ecological network analysis to the CBC  
456 targeting Common Sowthistle in Australia, the data reported here are potentially useful for other  
457 future programs.

## 458 Authors' contributions

459 Conceptualization: M. Ollivier, V. Lesieur, M. S. Tixier, J.-F. Martin

460 Methodology: M. Ollivier, J. Tavoillot, F. Bénétière, V. Lesieur, J.-F. Martin



461 Data acquisition: M. Ollivier, J. Tavoillot, F. Bénétière, V. Lesieur, J.-F. Martin

462 Data analysis: M. Ollivier

463 Supervision: M. S. Tixier, J.-F. Martin,

464 Writing original draft: M. Ollivier

465 Writing review and editing: M. Ollivier, J. Tavoillot, V. Lesieur, M. S. Tixier, J.-F. Martin

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475

## 476 Literature cited

- 477 Adkins, S.W., Wills, D., Boersma, M., Walker, S.R., Robinson, G., Mcleod, R.J., and Einam, J.P.  
478 (1997). Weeds resistant to chlorsulfuron and atrazine from the north-east grain region of Australia.  
479 *Weed Research* 37, 343–349.
- 480 Alberdi, A., Aizpurua, O., Gilbert, M.T.P., and Bohmann, K. (2018). Scrutinizing key steps for  
481 reliable metabarcoding of environmental samples. *Methods in Ecology and Evolution* 9, 134–147.
- 482 Aphids on the world's plants Database (2020). Aphids on the world's plants Database - An online  
483 identification and information guide.
- 484 Barratt, B.I.P., Moran, V.C., Bigler, F., and van Lenteren, J.C. (2018). The status of biological  
485 control and recommendations for improving uptake for the future. *BioControl* 63, 155–167.
- 486 Bellard, C., Genovesi, P., and Craine, J.M. (2016). Global patterns in threats to vertebrates by  
487 biological invasions. *Proceedings of the Royal Society B: Biological Sciences* 283, 20152454.
- 488 Benavent-Corai, J. (2005). Catalogue of the Hosts-plants of the World Agromyzidae (Diptera)  
489 (Università degli Studi di Milano).
- 490 Bersier, L.-F., Banašek-Richter, C., and Cattin, M.-F. (2002). Quantitative Descriptors of Food-  
491 Web Matrices. *Ecology* 83, 2394–2407.
- 492 Bladmineerders Online database (2020). Plant Parasites of Europe – leafminers, galls and fungi.
- 493 Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C., Al-Ghalith, G.A., Alexander,  
494 H., Alm, E.J., Arumugam, M., Asnicar, F., et al. (2018). QIIME 2: Reproducible, interactive,  
495 scalable, and extensible microbiome data science (PeerJ Inc.).
- 496 Boulos, L. (1974). Revision systematique du genre *Sonchus* L. s.l. VI. Sous-genre. 3. *origosonchus*.  
497 genres *Embergeria*, *Babcockia* et *-Taeckholmia*. species *exclusae* et *dubiae*. index. *Botaniska*  
498 *notiser*.
- 499 Brandon-Mong, G.-J., Gan, H.-M., Sing, K.-W., Lee, P.-S., Lim, P.-E., and Wilson, J.-J. (2015).  
500 DNA metabarcoding of insects and allies: an evaluation of primers and pipelines. *Bull. Entomol.*  
501 *Res.* 105, 717–727.
- 502 Braukmann, T.W.A., Kuzmina, M.L., Sills, J., Zakharov, E.V., and Hebert, P.D.N. (2017). Testing  
503 the Efficacy of DNA Barcodes for Identifying the Vascular Plants of Canada. *PLoS One* 12.
- 504 Briese, D.T. (2005). Translating host-specificity test results into the real world: The need to  
505 harmonize the yin and yang of current testing procedures. *Biological Control* 35, 208–214.
- 506 Buccellato, L., Byrne, M.J., Fisher, J.T., and Witkowski, E.T.F. (2019). Post-release evaluation of  
507 a combination of biocontrol agents on Crofton weed: testing extrapolation of greenhouse results to  
508 field conditions.

- 509 Burdfield-Steel, E.R., and Shuker, D.M. (2014). The evolutionary ecology of the Lygaeidae. *Ecol*  
510 *Evol* 4, 2278–2301.
- 511 Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. (2016).  
512 DADA2: High resolution sample inference from Illumina amplicon data. *Nat Methods* 13, 581–  
513 583.
- 514 Carvalheiro, L.G., Buckley, Y.M., Ventim, R., Fowler, S.V., and Memmott, J. (2008). Apparent  
515 competition can compromise the safety of highly specific biocontrol agents. *Ecology Letters* 11,  
516 690–700.
- 517 Ceglar, A., Zampieri, M., Toreti, A., and Dentener, F. (2019). Observed Northward Migration of  
518 Agro-Climate Zones in Europe Will Further Accelerate Under Climate Change. *Earth's Future* 7,  
519 1088–1101.
- 520 Centre de Biologie pour la Gestion des Population (2019). CBGP - Continental Arthropod  
521 Collection (Portail Data INRAE).
- 522 Chao, A. (1984). Nonparametric Estimation of the Number of Classes in a Population.  
523 *Scandinavian Journal of Statistics* 11, 265–270.
- 524 Chase, M.W., Christenhusz, M.J.M., Fay, M.F., Byng, J.W., Judd, W.S., Soltis, D.E., Mabberley,  
525 D.J., Sennikov, A.N., Soltis, P.S., and Stevens, P.F. (2016). An update of the Angiosperm  
526 Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Bot J Linn*  
527 *Soc* 181, 1–20.
- 528 Clare, E.L. (2014). Molecular detection of trophic interactions: emerging trends, distinct  
529 advantages, significant considerations and conservation applications. *Evolutionary Applications* 7,  
530 1144–1157.
- 531 Creedy, T.J., Ng, W.S., and Vogler, A.P. (2019). Toward accurate species-level metabarcoding of  
532 arthropod communities from the tropical forest canopy. *Ecology and Evolution* 9, 3105–3116.
- 533 Cruaud, A., Gonzalez, A.-A., Godefroid, M., Nidelet, S., Streito, J.-C., Thuillier, J.-M., Rossi, J.-  
534 P., Santoni, S., and Rasplus, J.-Y. (2018). Using insects to detect, monitor and predict the  
535 distribution of *Xylella fastidiosa*: a case study in Corsica. *Sci Rep* 8, 1–13.
- 536 Cullen, J., Julien, M., and McFadyen, R. (2012). *Biological Control of Weeds in Australia*.  
537 (Collingwood: CSIRO Publishing).
- 538 Derocles, S.A.P., Bohan, D.A., Dumbrell, A.J., Kitson, J.J.N., Massol, F., Pauvert, C.,  
539 Plantegenest, M., Vacher, C., and Evans, D.M. (2018). Chapter One - Biomonitoring for the 21st  
540 Century: Integrating Next-Generation Sequencing Into Ecological Network Analysis. In *Advances*  
541 *in Ecological Research*, D.A. Bohan, A.J. Dumbrell, G. Woodward, and M. Jackson, eds.  
542 (Academic Press), pp. 1–62.
- 543 Diegisser, T., Seitz, A., and Johannesen, J. (2006). Phylogeographic patterns of host-race evolution  
544 in *Tephritis conura* (Diptera: Tephritidae). *Molecular Ecology* 15, 681–694.

- 545 Diputacio Barcelona (2019). Trobada d'Estudiosos de Sant Llorenç del Munt i l'Obac -  
546 Comunicacions presentades el 21 i 22 de novembre de 2017 a Castellar del Vallès.
- 547 Erickson, D.L., Reed, E., Ramachandran, P., Bourg, N.A., McShea, W.J., and Ottesen, A. (2017).  
548 Reconstructing a herbivore's diet using a novel rbcL DNA mini-barcode for plants. *AoB PLANTS*  
549 *9*.
- 550 Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. (1994). DNA primers for  
551 amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan  
552 invertebrates. *Mol. Marine Biol. Biotechnol.* *3*, 294–299.
- 553 Fréchette, B., Rojo, S., Alomar, O., and Lucas, É. (2006). Intraguild predation between syrphids  
554 and mirids: who is the prey? Who is the predator? *BioControl* *52*, 175.
- 555 Frei, B., Guenay, Y., Bohan, D.A., Traugott, M., and Wallinger, C. (2019). Molecular analysis  
556 indicates high levels of carabid weed seed consumption in cereal fields across Central Europe. *J*  
557 *Pest Sci* *92*, 935–942.
- 558 Galan, M., Pons, J.-B., Tournayre, O., Pierre, É., Leuchtman, M., Pontier, D., and Charbonnel, N.  
559 (2017). Metabarcoding for the parallel identification of several hundred predators and their prey:  
560 Application to bat species diet analysis. *Molecular Ecology Resources* *18*, 474–489.
- 561 García-Robledo, C., Erickson, D.L., Staines, C.L., Erwin, T.L., and Kress, W.J. (2013). Tropical  
562 plant-herbivore networks: reconstructing species interactions using DNA barcodes. *PLoS One* *8*,  
563 e52967.
- 564 Garipey, T.D., Haye, T., and Zhang, J. (2014). A molecular diagnostic tool for the preliminary  
565 assessment of host-parasitoid associations in biological control programmes for a new invasive  
566 pest. *Mol. Ecol.* *23*, 3912–3924.
- 567 Groenteman, R., Fowler, S.V., and Sullivan, J.J. (2011). St. John's wort beetles would not have  
568 been introduced to New Zealand now: A retrospective host range test of New Zealand's most  
569 successful weed biocontrol agents. *Biological Control* *57*, 50–58.
- 570 Haye, T., Achterberg, C. van, Goulet, H., Barratt, B.I.P., and Kuhlmann, U. (2006). Potential for  
571 classical biological control of the potato bug *Closterotomus norwegicus* (Hemiptera: Miridae):  
572 description, parasitism and host specificity of *Peristenus closterotomae* sp. n. (Hymenoptera:  
573 Braconidae). *Bulletin of Entomological Research* *96*, 421–431.
- 574 Hinz, H.L., Winston, R.L., and Schwarzländer, M. (2019). How Safe Is Weed Biological Control?  
575 A Global Review of Direct Nontarget Attack. *The Quarterly Review of Biology* *94*, 1–27.
- 576 Hinz, H.L., Winston, R.L., and Schwarzländer, M. (2020). A global review of target impact and  
577 direct nontarget effects of classical weed biological control. *Current Opinion in Insect Science* *38*,  
578 48–54.

- 579 Hosseini, R., Schmidt, O., and Keller, M.A. (2008). Factors affecting detectability of prey DNA in  
580 the gut contents of invertebrate predators: a polymerase chain reaction-based method. *Entomologia*  
581 *Experimentalis et Applicata* 126, 194–202.
- 582 Hrček, J., and Godfray, H.C.J. (2015). What do molecular methods bring to host–parasitoid food  
583 webs? *Trends in Parasitology* 31, 30–35.
- 584 Hutchinson, I., Colosi, J., and Lewin, R.A. (1984). THE BIOLOGY OF CANADIAN WEEDS.:  
585 63. *Sonchus asper* (L.) Hill and *S. oleraceus* L. *Can. J. Plant Sci.* 64, 731–744.
- 586 Jalaludin, A., Widderick, M.J., Broster, J., and Walsh, M.J. (2018). Glyphosate and 2,4-D amine  
587 resistance in common sowthistle (*Sonchus oleraceus*) and fleabane (*Conyza bonariensis*) in the  
588 northern grain growing region of Australia. p.
- 589 Jordano, P. (2016). Sampling networks of ecological interactions. *Functional Ecology* 30, 1883–  
590 1893.
- 591 Keane, R.M., and Crawley, M.J. (2002). Exotic plant invasions and the enemy release hypothesis.  
592 *Trends in Ecology & Evolution* 17, 164–170.
- 593 Kennedy, S.R., Prost, S., Overcast, I., Rominger, A.J., Gillespie, R.G., and Krehenwinkel, H.  
594 (2020). High-throughput sequencing for community analysis: the promise of DNA barcoding to  
595 uncover diversity, relatedness, abundances and interactions in spider communities. *Dev Genes Evol*  
596 230, 185–201.
- 597 Landolt, P.J., Worth, R.A., and Zack, R.S. (2010). First Report of *Hecatera dysodea* (Denis and  
598 Schiffermüller) (Noctuidae) in the Pacific Northwest of the United States. *Journal of the*  
599 *Lepidopterists' Society* 64, 192–196.
- 600 Leray, M., Yang, J.Y., Meyer, C.P., Mills, S.C., Agudelo, N., Ranwez, V., Boehm, J.T., and  
601 Machida, R.J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial  
602 COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut  
603 contents. *Frontiers in Zoology* 10, 34.
- 604 Lesieur, V., Thomann, T., Ollivier, M., and Raghu, S. (2020). Making host specificity testing more  
605 efficient: Exploring the use of abridged test plant lists. *Journal of Applied Entomology* 144, 546–  
606 551.
- 607 Lesieur et al. in prep. Feasibility of classical biological control of *Sonchus oleraceus* in Australia,  
608 *Biocontrol Science and Technology*.
- 609 Lewinsohn, T.M., Prado, P.I., Jordano, P., Bascompte, J., and Olesen, J.M. (2006). Structure in  
610 plant–animal interaction assemblages. *Oikos* 113, 174–184.
- 611 Llewellyn, R., Ronning, D., Ouzman, J., Walker, S., Mayfield, A., and Clarke, M. (2016). Impact  
612 of weeds on Australian grain production : the cost of weeds to Australian grain growers and the  
613 adoption of weed management and tillage practices (GRDC).

- 614 López-Núñez, F.A., Heleno, R.H., Ribeiro, S., Marchante, H., and Marchante, E. (2017). Four-  
615 trophic level food webs reveal the cascading impacts of an invasive plant targeted for biocontrol.  
616 *Ecology* 98, 782–793.
- 617 Louda, S.M., Kendall, D., Connor, J., and Simberloff, D. (1997). Ecological Effects of an Insect  
618 Introduced for the Biological Control of Weeds. *Science* 277, 1088–1090.
- 619 Louda, S.M., Pemberton, R.W., Johnson, M.T., and Follett, P.A. (2003). Nontarget effects--the  
620 Achilles' heel of biological control? Retrospective analyses to reduce risk associated with  
621 biocontrol introductions. *Annu. Rev. Entomol.* 48, 365–396.
- 622 Macías-Hernández, N., Athey, K., Tonzo, V., Wangenstein, O.S., Arnedo, M., and Harwood, J.D.  
623 (2018). Molecular gut content analysis of different spider body parts. *PLoS One* 13, e0196589.
- 624 Marquina, D., Andersson, A.F., and Ronquist, F. (2019). New mitochondrial primers for  
625 metabarcoding of insects, designed and evaluated using in silico methods. *Molecular Ecology*  
626 *Resources* 19, 90–104.
- 627 Martin, J.-F. (2019). error-proof\_indexes v1.0 (Zenodo). <http://doi.org/10.5281/zenodo.3350207>
- 628 McFadyen, R.E. (1998). Biological control of weeds. *Annu. Rev. Entomol.* 43, 369–393.
- 629 Memmott, J. (2000). Food webs as a tool for studying nontarget effects in biological control. In  
630 *Nontarget Effects of Biological Control*, P.A. Follett, and J.J. Duan, eds. (Boston, MA: Springer  
631 US), pp. 147–163.
- 632 Memmott, J. (2009). Food webs: a ladder for picking strawberries or a practical tool for practical  
633 problems? *Philos Trans R Soc Lond B Biol Sci* 364, 1693–1699.
- 634 Meulen, A.W. van der, Widderick, M., Cook, T., Chauhan, B.S., and Bell, K. (2016). Survey of  
635 glyphosate resistance in common sowthistle (*Sonchus oleraceus*) across the Australian Northern  
636 Grains Region. In *20th Australasian Weeds Conference*, (Perth, Western Australia), p. 108.
- 637 Morin, L., Reid, A.M., Sims-Chilton, N.M., Buckley, Y.M., Dhileepan, K., Hastwell, G.T.,  
638 Nordblom, T.L., and Raghu, S. (2009). Review of approaches to evaluate the effectiveness of weed  
639 biological control agents. *Biological Control* 51, 1–15.
- 640 Müller-Schärer, H., and Schaffner, U. (2008). Classical biological control: exploiting enemy  
641 escape to manage plant invasions. *Biol Invasions* 10, 859–874.
- 642 Ollivier, M., Lesieur, V., Raghu, S., and Martin, J.-F. (2020). Characterizing ecological interaction  
643 networks to support risk assessment in classical biological control of weeds. *Current Opinion in*  
644 *Insect Science* 38, 40–47.
- 645 Oksanen J., Blanchet F. G., Friendly M., Kindt R., Legendre P., McGlenn D., Minchin P R., O'Hara  
646 R. B., Simpson G. L., Solymos P., Stevens M. H. H., Szoecs E. and Wagner H. (2019). vegan:  
647 Community Ecology Package. R package version 2.5-6. [https://CRAN.R-](https://CRAN.R-project.org/package=vegan)  
648 [project.org/package=vegan](https://CRAN.R-project.org/package=vegan)

- 649 Paynter, Q., Fowler, S.V., and Groenteman, R. (2017). Making weed biological control predictable,  
650 safer and more effective: perspectives from New Zealand. *BioControl* 1–10.
- 651 Pearson, D.E., and Callaway, R.M. (2003). Indirect effects of host-specific biological control  
652 agents. *Trends in Ecology & Evolution* 18, 456–461.
- 653 Peterson, P.G., Merrett, M.F., Fowler, S.V., Barrett, D.P., and Paynter, Q. (2020). Comparing  
654 biocontrol and herbicide for managing an invasive non-native plant species: Efficacy, non-target  
655 effects and secondary invasion. *Journal of Applied Ecology* 57, 1876–1884.
- 656 Pyšek, P., Pergl, J., Essl, F., Lenzner, B., Dawson, W., Kreft, H., Weigelt, P., Winter, M., Kartesz,  
657 J., Nishino, M., et al. (2017). Naturalized alien flora of the world: species diversity, taxonomic and  
658 phylogenetic patterns, geographic distribution and global hotspots of plant invasion. *Preslia* 89,  
659 203–274.
- 660 Ratnasingham, S., and Hebert, P.D.N. (2007). bold: The Barcode of Life Data System  
661 (<http://www.barcodinglife.org>). *Mol Ecol Notes* 7, 355–364.
- 662 Rizzo, M.C., and Massa, B. (1998). On two Italian gall midges (Diptera Cecidomyiidae) and their  
663 parasitoids. *Entomologica* 32, 121–131.
- 664 Roslin, T., and Majaneva, S. (2016). The use of DNA barcodes in food web construction-terrestrial  
665 and aquatic ecologists unite! *Genome* 59, 603–628.
- 666 Schmid, U., and Grossmann, A. (1996). Eiablage von *Cheilosia latifrons* (Zetterstedt,  
667 1843)(Diptera, Syrphidae) an *Leontodón autumnalis* L. *Volucella* 2, 86–87.
- 668 Schoonhoven, L.M., Jermy, T., and Loon, J.J.A. van (1998). *Insect-plant biology: from physiology  
669 to evolution*. (London: Chapman and Hall).
- 670 Seebens, H., Essl, F., Dawson, W., Fuentes, N., Moser, D., Pergl, J., Pyšek, P., Kleunen, M. van,  
671 Weber, E., Winter, M., et al. (2015). Global trade will accelerate plant invasions in emerging  
672 economies under climate change. *Global Change Biology* 21, 4128–4140.
- 673 Sheppard, S.K., and Harwood, J.D. (2005). Advances in molecular ecology: tracking trophic links  
674 through predator–prey food-webs. *Functional Ecology* 19, 751–762.
- 675 Sheppard, S.K., Henneman, M.L., Memmott, J., and Symondson, W.O.C. (2004). Infiltration by  
676 alien predators into invertebrate food webs in Hawaii: a molecular approach. *Mol Ecol* 13, 2077–  
677 2088.
- 678 Smit, J., Reijnen, B., and Stokvis, F. (2013). Half of the European fruit fly species barcoded  
679 (Diptera, Tephritidae); a feasibility test for molecular identification. *Zookeys* 279–305.
- 680 Speight, M.C.D. (2014). Species accounts of European Syrphidae (Diptera), 2014. 319.
- 681 Suckling, D.M., and Sforza, R.F.H. (2014). What Magnitude Are Observed Non-Target Impacts  
682 from Weed Biocontrol? *PLOS ONE* 9, e84847.

- 683 Tay, E.B. (1972). Population Ecology of *Cicadella Viridis* (L.) and Bionomics of *Graphocephala*  
684 *Coccinea* (Forster)(Homoptera, Cicadellidae). (University of London (Imperial College of Science  
685 and Technology)).
- 686 Thébault, E., and Fontaine, C. (2010). Stability of Ecological Communities and the Architecture  
687 of Mutualistic and Trophic Networks. *Science* 329, 853–856.
- 688 Todd, J.H., Pearce, B.M., and Barratt, B.I.P. (2020). Using qualitative food webs to predict species  
689 at risk of indirect effects from a proposed biological control agent. *BioControl*.
- 690 Van Driesche, R.G., Carruthers, R.I., Center, T., Hoddle, M.S., Hough-Goldstein, J., Morin, L.,  
691 Smith, L., Wagner, D.L., Blossey, B., Brancatini, V., et al. (2010). Classical biological control for  
692 the protection of natural ecosystems. *Biological Control* 54, S2–S33.
- 693 Vázquez Albalade, X. (2002). European fauna of Oedemeridae: Coleoptera (Barcelona: Argania  
694 Ed).
- 695 Veldtman, R., Lado, T.F., Botes, A., Procheş, Ş., Timm, A.E., Geertsema, H., and Chown, S.L.  
696 (2011). Creating novel food webs on introduced Australian acacias: indirect effects of galling  
697 biological control agents. *Diversity and Distributions* 17, 958–967.
- 698 Vitousek, P.M., Mooney, H.A., Lubchenco, J., and Melillo, J.M. (1997). Human Domination of  
699 Earth's Ecosystems. *Science* 277, 494–499.
- 700 Wang, Q. (2017). *Cerambycidae of the World: Biology and Pest Management* (CRC Press).
- 701 Wapshere, A.J., Delfosse, E.S., and Cullen, J.M. (1989). Recent developments in biological control  
702 of weeds. *Crop Protection* 8, 227–250.
- 703 Warren, P.H. (1994). Making connections in food webs. *Trends in Ecology & Evolution* 9, 136–  
704 141.
- 705 Wheeler, A.G. (2001). Biology of the plant bugs (Hemiptera: Miridae): pests, predators,  
706 opportunists. *Biology of the Plant Bugs (Hemiptera: Miridae): Pests, Predators, Opportunists*.
- 707 White, I.M. (1988). Tephritid flies: Diptera: Tephritidae. *Tephritid Flies: Diptera: Tephritidae*. 10.
- 708 Widderick, M.J., Walker, S.R., Sindel, B.M., and Bell, K.L. (2010). Germination, emergence, and  
709 persistence of *Sonchus oleraceus*, a major crop weed in subtropical Australia. *Weed Biology and*  
710 *Management* 10, 102–112.
- 711 Willis, A.J., and Memmott, J. (2005). The potential for indirect effects between a weed, one of its  
712 biocontrol agents and native herbivores: A food web approach. *Biological Control* 35, 299–306.
- 713 Wirta, H.K., Hebert, P.D.N., Kaartinen, R., Prosser, S.W., Várkonyi, G., and Roslin, T. (2014).  
714 Complementary molecular information changes our perception of food web structure. *PNAS* 111,  
715 1885–1890.



716 Zhu, C., Gravel, D., and He, F. (2019). Seeing is believing? Comparing plant–herbivore networks  
717 constructed by field co-occurrence and DNA barcoding methods for gaining insights into network  
718 structures. *Ecology and Evolution* 9, 1764–1776.

719

720 **Tables**

721

722 *Table 1: Global description of the meta-network and subnetwork centred on S. oleraceus. Metrics measured are the*  
 723 *total number of nodes (in brackets is the number of nodes connected in the network), the number of links, the linkage*  
 724 *density and the connectance of the network. For each network is also presented the number of species per trophic level*  
 725 *(in brackets is the number of species in interaction with another species). Omnivorous species are regarded as natural*  
 726 *enemies.*

		<b>Meta-network</b>	<b>Subnetwork*</b>
<b>Global metrics</b>	Nb of nodes (connected)	401 (241)	116
	Nb of links	350	213
	Linkage density	1,45	1,84
	Connectance	0,006	0,008
<b>Taxon assemblage</b>	Nb of plant species (in interaction)	132 (60)	39
	Nb of herbivore species (in interaction)	185 (136)	47
	Nb of natural enemies species (in interaction)	79 (35)	30

\* All nodes are connected

727

728 Table 2: Herbivores from *Sonchus oleraceus* (SO) collected and identified through intense field sampling in three bioclimatic regions  
 729 (Semi-oceanic, Mediterranean and Continental) in France in spring 2018. The field host range of these herbivores was defined by  
 730 network analysis (S - SO: Specific to SO, S – S: Specific to the genus *Sonchus*, S – C: Specific to the tribe Cichorieae, G: Generalist,  
 731 ? : Unknown host range). The column 3-years surveys refers to the species collected following classical procedures of biocontrol  
 732 program (see Materials and Methods section).

733

Order / Family	Species	Trophic guild	3-years survey	Field host range	Literature host range (Reference)
<b>Coleoptera</b>					
Cerambycidae	<i>Agapanthia cardui</i> (Linnaeus, 1767)	Stem boring	No	G	
Cerambycidae	<i>Agapanthia pannonica</i> Kratochvil, 1985	Stem boring	No	S - SO	Polyphagous (Wang, 2017)
Curculionidae	<i>sp.</i>	Crown/stem boring	–	G	
Curculionidae	<i>Lixus punctiventris</i> (Boheman, 1835)	Stem boring	Yes	S - C	
Dasytidae	<i>Dasytes tristiculus</i> Mulsant & Rey, 1868	Flower/pollen chewing	No	S - SO	Polyphagous (Diputacio Barcelona, 2019)
Dasytidae	<i>Psilothrix viridicoeruleus</i> (Geoffroy, 1785)	Flower/pollen chewing	No	G	
Oedemeridae	<i>Oedemera crassipes</i> Ganglbauer, 1881)	Flower/pollen chewing	No	S - SO	Polyphagous (Vázquez Albalate, 2002)
Oedemeridae	<i>Oedemera flavipes</i> (Fabricius, 1792)	Flower/pollen chewing	No	G	
Nitidulidae	<i>Brassicogethes aeneus</i> (Fabricius, 1775)	Flower/pollen chewing	Yes	G	
<b>Diptera</b>					
Agromyzidae	<i>sp.</i>	Leaf mining	–	G	
Agromyzidae	<i>Liriomyza sonchi</i> Hendel, 1931	Leaf mining	Yes	S - S	Oligophagous (Benavent-Corai, 2005)
Agromyzidae	<i>Ophiomyia cunctata</i> (Hendel, 1920)	Leaf mining	Yes	S - C	
Agromyzidae	<i>Phytomyza horticola</i> Goureau, 1851	Leaf mining	Yes	G	
Agromyzidae	<i>Phytomyza lateralis</i> Fallén, 1823	Leaf mining	Yes	S - C	
Tephritidae	<i>sp.</i>	Flower bud galling/seed feeding	–	S - SO	Oligophagous (White, 1988)
Tephritidae	<i>Campiglossa producta</i> (Loew, 1844)	Flower bud galling/seed feeding	Yes	S - C	
Tephritidae	<i>Ensina sonchi</i> (Linnaeus, 1767)	Flower bud galling/seed feeding	Yes	S - S	Oligophagous (White, 1988)
Tephritidae	<i>Tephritis sp.</i>	Flower bud galling/seed feeding	–	G	
Tephritidae	<i>Tephritis cometa</i> (Loew, 1840)	Flower bud galling/seed feeding	No	S - SO	Oligophagous (Bladmineerders Online database, 2020)
Tephritidae	<i>Tephritis formosa</i> (Loew, 1844)	Flower bud galling/seed feeding	Yes	S - C	

Tephritidae	<i>Tephritis vespertina</i> (Loew, 1844)	Flower bud galling/seed feeding	No	S - SO	Oligophagous (Bladmineerders Online database, 2020)
<b>Hemiptera</b>					
Aphididae	<i>sp.</i>	Systemic sucking piercing	_	S - SO	Polyphagous (Aphids on the world's plants Database, 2020)
Aphididae	<i>Aphis craccivora</i> Koch, 1854	Systemic sucking piercing	No	S - C	
Aphididae	<i>Aphis fabae</i> Scopoli, 1763	Systemic sucking piercing	No	G	
Aphididae	<i>Hyalopterus pruni</i> (Geoffroy, 1762)	Systemic sucking piercing	No	S - SO	Oligophagous (Aphids on the world's plants Database, 2020)
Aphididae	<i>Hyperomyzus lactucae</i> (Linnaeus, 1758)	Systemic sucking piercing	Yes	G	
Aphididae	<i>Macrosiphum rosae</i> (Linnaeus, 1758)	Systemic sucking piercing	No	S - SO	Polyphagous (Aphids on the world's plants Database, 2020)
Aphididae	<i>Uroleucon sonchi</i> (Linnaeus, 1767)	Systemic sucking piercing	Yes	G	
Anthocoridae	<i>Orius sp.</i>	Flower head sucking piercing	No	G	
Aphrophoridae	<i>Philaenus sp.</i>	Leaf sucking piercing	No	G	
Aphrophoridae	<i>Philaenus spumarius</i> (Linnaeus, 1758)	Leaf sucking piercing	Yes	G	
Cicadellidae	<i>Cicadella viridis</i> (Linnaeus, 1758)	Leaf sucking piercing	No	S - SO	Polyphagous (Tay, 1972)
Coreidae	<i>Coreus marginatus</i> (Linnaeus, 1758)	Flower head sucking piercing	Yes	G	
Lygaeidae	<i>Lygaeus equestris</i> (Linnaeus, 1758)	Flower head sucking piercing	Yes	G	
Miridae	<i>sp.</i>	Flower head sucking piercing	_	G	
Miridae	<i>Closterotomus norvegicus</i> (Gmelin, 1790)	Flower head sucking piercing	No	S - SO	Polyphagous (Haye et al., 2006)
Miridae	<i>Lepidargyrus ancorifer</i> (Fieber, 1858)	Flower head sucking piercing	No	G	
Orsillidae	<i>Nysius cymoides</i> (Spinola, 1837)	Flower head sucking piercing	Yes	G	
Pentatomidae	<i>Dolycoris baccarum</i> (Linnaeus, 1758)	Flower head sucking piercing	Yes	G	
Pentatomidae	<i>Nezara viridula</i> (Linnaeus, 1758)	Flower head sucking piercing	No	G	
Rhopalidae	<i>Liorhyssus hyalinus</i> (Fabricius, 1794)	Flower head sucking piercing	Yes	G	
Rhopalidae	<i>Stictopleurus punctatonervosus</i> (Goeze, 1778)	Flower head sucking piercing	Yes	G	
<b>Hymenoptera</b>					
Cynipidae	<i>sp.</i>	Stem boring	Yes	G	
Tenthredinidae	<i>Cephaledo bifasciata</i> (Müller, 1766)	Leaf chewing	No	S - SO	
<b>Lepidoptera</b>					
Noctuidae	<i>Hecatera dysodea</i> (Denis & Schiffermüller, 1775)	Flower bud chewing	Yes	S - SO	Oligophagous (Landolt et al., 2010)
Tortricidae	<i>sp.</i>	Leaf mining/chewing	_	S - SO	Polyphagous (Bladmineerders Online database, 2020)
Tortricidae	<i>Cnephasia stephensiana</i> (Doubleday, 1849)	Leaf mining/chewing	No	S - SO	Polyphagous (Bladmineerders Online database, 2020)

735 **Figures**

736



*Figure 1: Accumulation curves representing a) species richness in plants and arthropods and b) pairwise interactions from the meta-network and focusing on interactions involving *S. oleraceus*. Curves were constructed with 1000 random resampling events over the 57 quadrats analyzed along the sampling campaign (Spring 2018).*

737

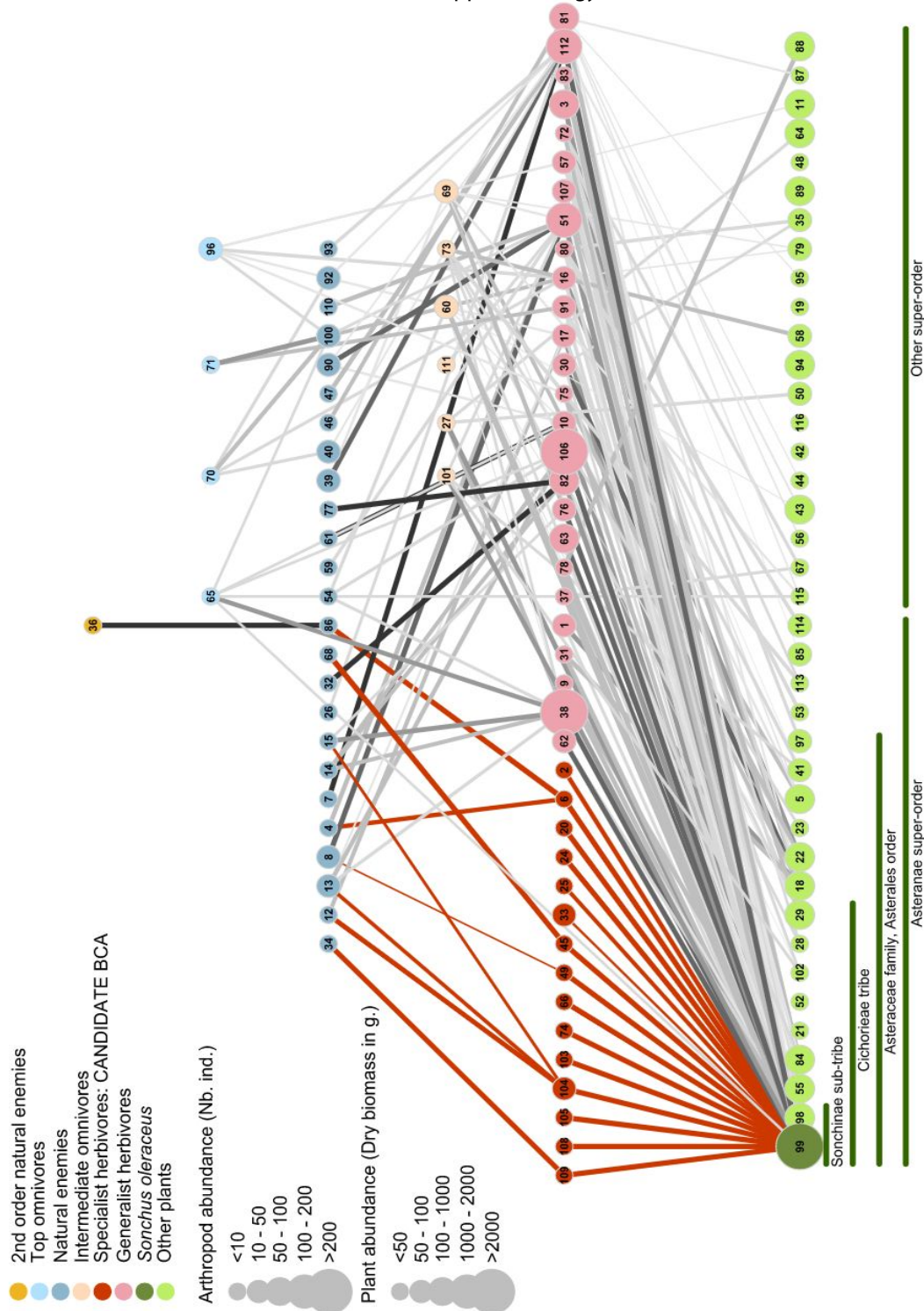


Figure 2: Multitrophic network reconstructed from *S. oleraceus* (dark green node) and 38 other plants (light green nodes) used by *S. oleraceus* herbivores (red nodes represent putative specialist herbivores, and pink nodes correspond to herbivores feeding on other species in addition to *S. oleraceus*). Plants are ordered by their phylogenetic relatedness to *S. oleraceus*. Natural enemies of herbivore species are represented by dark blue nodes. Nodes at intermediate levels (beige and light blue) correspond to omnivorous species identified by molecular analyses. The width of edges reflects frequencies of interactions between pairs of species and edges coloured in red emphasize interactions involving potential candidate BCA. The list of taxa corresponding to each node and the edge list are provided in Supplementary Tables 3 and 4, respectively.



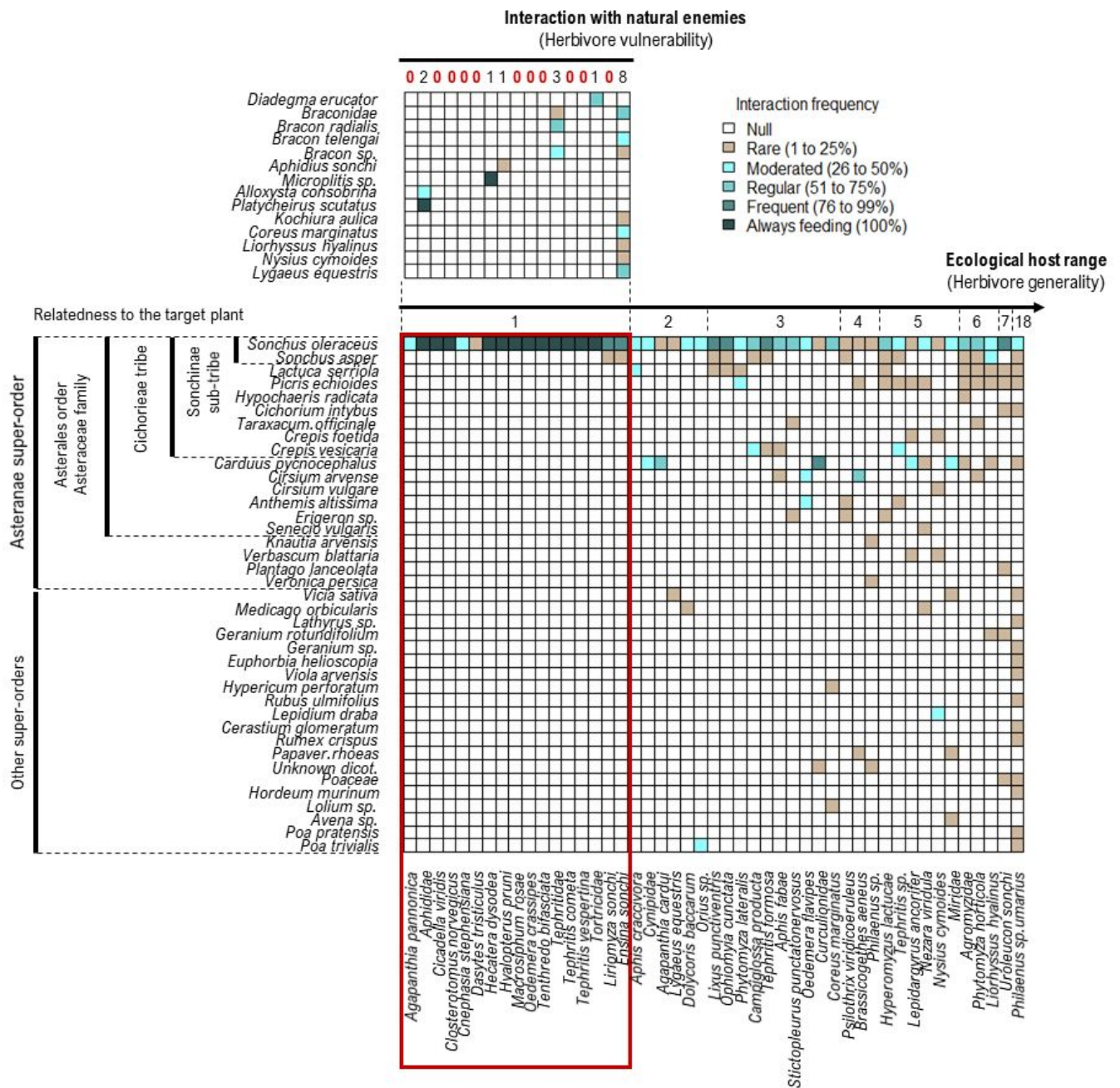


Figure 3: Interaction matrix between herbivores sampled on *Sonchus oleraceus* and their resource plants, indicating the ecological host range of the herbivores, as defined by intense field sampling in France, during spring 2018. Plants are ordered by their phylogenetic relatedness to *S. oleraceus* and arthropods are ordered by increasing generality values (i.e. the number of resources per species). Red rectangle highlights the 17 species considered as candidate BCA for their restricted ecological host range. The second level matrix displays interactions between candidate BCA and their natural enemies. Interaction are represented as semi-quantitative information, via occurrences frequencies of interactions culculated for each species pair.

## Supplementary materials

[Supplementary Texts](#)

[Supplementary Tables](#)

[Supplementary Figures](#)

[References](#)



## Supplementary Texts

### *Text 1: Curation and transformation of the interaction matrix*

In order to produce an interaction matrix, in which observations are resources and variables are consumers, the original dataset should be checked. First, for each sample, the taxa having the highest number of reads were set as the consumer. This assumption was confirmed in most of the cases (the specimen collected in the tube was the consumer and produced the highest number of reads). However due to the simultaneous detection of possible preys and parasitoids from the specimen sampled and the contrasted amplification rates between taxonomic groups (Alberdi et al. 2018), this was not always the case. Consequently, a second step of data manual validation was required on each sample (Supplementary Figure S2) in order to 1) verify whether the consumer was correctly defined according to the observation from the field, 2) add the source plant for herbivorous arthropods, 3) define the status of the taxa simultaneously amplified with the consumer (prey, parasitoid or contamination). The next step consisted in removing cannibalistic interactions (i. e. delete reads at the intersection of a same source and consumer species). Indeed, DNA-based methods are not suitable to disentangle predator and prey sequences (Taberlet et al. 2018). As metabarcoding neither provides quantitative information about the trophic links (Deagle et al. 2019), the numbers of reads were converted to binary information, then occurrence frequencies of interactions between a pair of consumer/prey species were calculated as the percentage of consumer samples positive for the prey over the total number of consumer sampled in the dataset. All analyses were carried out under R version 3.5.2 (R Core Team 2018).

## Supplementary Tables

Table 1: Locations of the different sites prospected per sampling session in the three bioclimatic regions.

	Site	Latitude	Longitude	Environment
<b>Semi-oceanic</b>				
<i>Session 1</i>	Laurabuc	43.26462	1.99578	Agricultural
	Saint-Martin-Lalande	43.29428	1.99238	Agricultural
	Castelnaudary	43.33395	1.99262	Agricultural
<i>Session 2</i>	Laurabuc	43.26462	1.99578	Agricultural
	Saint-Martin-Lalande	43.29428	1.99238	Agricultural
<i>Session 3</i>	Laurabuc	43.26462	1.99578	Agricultural
	Villasavary	43.23643	2.03641	Ruderal
<b>Mediterranean</b>				
<i>Session 1</i>	La Valsière	43.65123	3.83356	Ruderal
	La Valsière	43.65123	3.83356	Ruderal
<i>Session 2</i>	Mauguio	43.6069	3.98868	Agricultural
	La Valsière	43.65123	3.83356	Ruderal
<i>Session 3</i>	Mauguio	43.6069	3.98868	Agricultural
	La Valsière	43.65123	3.83356	Ruderal
<b>Continental</b>				
<i>Session 1</i>	Mornant	45.60496	4.68696	Agricultural
	Saint-Génis-Laval	45.6909	4.76503	Agricultural
<i>Session 2</i>	Saint Laurent d'Agny	45.6335	4.6831	Agricultural
	Le Clair	45.63451	4.69026	Ruderal
<i>Session 3</i>	Saint Laurent d'Agny	45.6335	4.6831	Agricultural
	Thurigneux	45.59188	4.64755	Ruderal

Table 2: Detailed number of reads obtained from high-throughput sequencing for each of the COI markers employed

Marker	Raw reads	Filtered reads	Percentage of reads retained	Variants 2%
<b>HEX</b>	9083407	1205146	0,133	408
<b>HCO</b>	48226250	7190702	0,149	847
<b>LEP</b>	50173753	12320157	0,246	759
<b>Total</b>	<b>107483410</b>	<b>20716005</b>	<b>0,193</b>	<b>2014</b>

Table 3: List of the taxa included in the sub-network (Figure 2)

Node ID	Taxon	Order (Family)	Trophic level
1	<i>Agapanthia cardui</i>	Coleoptera (Cerambycidae)	Herbivore
2	<i>Agapanthia pannonica</i>	Coleoptera (Cerambycidae)	Herbivore
3	<i>Agromyzidae</i>	Diptera (Agromyzidae)	Herbivore
4	<i>Alloxysta consobrina</i>	Hymenoptera (Figitidae)	Natural enemy (parasitoid)
5	<i>Anthemis altissima</i>	Asterales (Asteraceae)	Plant
6	<i>Aphididae</i>	Hemiptera (Aphididae)	Herbivore
7	<i>Aphidius ervi</i>	Hemiptera (Aphididae)	Herbivore
8	<i>Aphidius sonchi</i>	Hemiptera (Aphididae)	Herbivore
9	<i>Aphis craccivora</i>	Hemiptera (Aphididae)	Herbivore
10	<i>Aphis fabae</i>	Hemiptera (Aphididae)	Herbivore
11	<i>Avena sp.</i>	Poales (Poaceae)	Plant
12	<i>Bracon radialis</i>	Hymenoptera (Braconidae)	Natural enemy (parasitoid)
13	<i>Bracon sp.</i>	Hymenoptera (Braconidae)	Natural enemy (parasitoid)
14	<i>Bracon telengai</i>	Hymenoptera (Braconidae)	Natural enemy (parasitoid)
15	<i>Braconidae</i>	Hymenoptera (Braconidae)	Natural enemy (parasitoid)
16	<i>Brassicogethes aeneus</i>	Coleoptera (Nitidulidae)	Herbivore
17	<i>Campiglossa producta</i>	Diptera (Tephritidae)	Herbivore
18	<i>Carduus pycnocephalus</i>	Asterales (Asteraceae)	Plant
19	<i>Cerastium glomeratum</i>	Caryophyllales (Caryophyllaceae)	Plant
20	<i>Cicadella viridis</i>	Hemiptera (Cicadellidae)	Herbivore
21	<i>Cichorium intybus</i>	Asterales (Asteraceae)	Plant
22	<i>Cirsium arvense</i>	Asterales (Asteraceae)	Plant
23	<i>Cirsium vulgare</i>	Asterales (Asteraceae)	Plant
24	<i>Closterotomus norvegicus</i>	Hemiptera (Miridae)	Herbivore
25	<i>Cnephasia stephensiana</i>	Lepidoptera (Tortricidae)	Herbivore
26	<i>Coccinella septempunctata</i>	Coleoptera (Coccinellidae)	Natural enemy (predator)
27	<i>Coreus marginatus</i>	Hemiptera (Coreidae)	Herbivore
28	<i>Crepis foetida</i>	Asterales (Asteraceae)	Plant
29	<i>Crepis vesicaria</i>	Asterales (Asteraceae)	Plant
30	<i>Curculionidae</i>	Coleoptera (Curculionidae)	Herbivore
31	<i>Cynipidae</i>	Hymenoptera (Cynipidae)	Herbivore
32	<i>Dacnusa maculipes</i>	Hymenoptera (Braconidae)	Natural enemy (parasitoid)
33	<i>Dasytes tristiculus</i>	Coleoptera (Melyridae)	Herbivore
34	<i>Diadegma erucator</i>	Hymenoptera (Ichneumonidae)	Natural enemy (parasitoid)
35	<i>Dicot unknown</i>	/	Plant
36	<i>Diplazon tibiatorius</i>	Hymenoptera (Ichneumonidae)	Natural enemy (parasitoid)
37	<i>Dolycoris baccarum</i>	Hemiptera (Pentatomidae)	Herbivore
38	<i>Ensina sonchi</i>	Diptera (Tephritidae)	Herbivore
39	<i>Ephedrus sp.</i>	Hymenoptera (Braconidae)	Natural enemy (parasitoid)
40	<i>Episyrphus balteatus</i>	Diptera (Syrphidae)	Natural enemy (predator)
41	<i>Erigeron sp.</i>	Asterales (Asteraceae)	Plant

42	<i>Euphorbia helioscopia</i>	Malpighiales (Euphorbiceae)	Plant
43	<i>Geranium rotundifolium</i>	Geraniales (Geraniaceae)	Plant
44	<i>Geranium sp.</i>	Geraniales (Geraniaceae)	Plant
45	<i>Hecatera dysodea</i>	Lepidoptera (Noctuidae)	Herbivore
46	<i>Heliophanus cupreus</i>	Araneae (Saltividae)	Natural enemy (predator)
47	<i>Heliophanus sp.</i>	Araneae (Saltividae)	Natural enemy (predator)
48	<i>Hordeum murinum</i>	Poales (Poaceae)	Plant
49	<i>Hyalopterus pruni</i>	Hemiptera (Aphididae)	Herbivore
50	<i>Hypericum perforatum</i>	Theales (Clusiaceae)	Plant
51	<i>Hyperomyzus lactucae</i>	Hemiptera (Aphididae)	Herbivore
52	<i>Hypochaeris radicata</i>	Asterales (Asteraceae)	Plant
53	<i>Knautia arvensis</i>	Dipsacales (Dipsacaceae)	Plant
54	<i>Kochiura aulica</i>	Araneae (Theridiidae)	Natural enemy (predator)
55	<i>Lactuca serriola</i>	Asterales (Asteraceae)	Plant
56	<i>Lathyrus sp.</i>	Fabales (Fabaceae)	Plant
57	<i>Lepidargyrus ancorifer</i>	Hemiptera (Miridae)	Herbivore
58	<i>Lepidium draba</i>	Capparales (Brassicaceae)	Plant
59	<i>Leptophyes punctatissima</i>	Orthoptera (Tettigoniidae)	Natural enemy (predator)
60	<i>Liorhyssus hyalinus</i>	Hemiptera (Rhopalidae)	Omnivore
61	<i>Lipolexis gracilis</i>	Hymenoptera (Braconidae)	Natural enemy (parasitoid)
62	<i>Liriomyza sonchi</i>	Diptera (Agromyzidae)	Herbivore
63	<i>Lixus punctiventris</i>	Coleoptera (Curculionidae)	Herbivore
64	<i>Lolium sp.</i>	Poales (Poaceae)	Plant
65	<i>Lygaeus equestris</i>	Hemiptera (Lygaeidae)	Omnivore
66	<i>Macrosiphum rosae</i>	Hemiptera (Aphididae)	Herbivore
67	<i>Medicago orbicularis</i>	Fabales (Fabaceae)	Plant
68	<i>Microplitis sp.</i>	Hymenoptera (Braconidae)	Natural enemy (parasitoid)
69	<i>Miridae</i>	Hemiptera (Miridae)	Omnivore
70	<i>Misumena vatia</i>	Araneae (Thomisidae)	Omnivore
71	<i>Neoscona adianta</i>	Araneae (Araneidae)	Omnivore
72	<i>Nezara viridula</i>	Hemiptera (Pentatomidae)	Herbivore
73	<i>Nysius cymoides</i>	Hemiptera (Orsillidae)	Omnivore
74	<i>Oedemera crassipes</i>	Coleoptera (Oedemeridae)	Herbivore
75	<i>Oedemera flavipes</i>	Coleoptera (Oedemeridae)	Herbivore
76	<i>Ophiomyia cunctata (beckeri)</i>	Diptera (Agromyzidae)	Herbivore
77	<i>Opiinae</i>	Hymenoptera (Braconidae)	Natural enemy (parasitoid)
78	<i>Orius sp.</i>	Hemiptera (Anthocoridae)	Herbivore
79	<i>Papaver rhoeas</i>	Ranunculales (Papaveraceae)	Plant
80	<i>Philaenus sp.</i>	Hemiptera (Aphrophoridae)	Herbivore
81	<i>Philaenus spumarius</i>	Hemiptera (Aphrophoridae)	Herbivore
82	<i>Phytomyza horticola (syngenesiae)</i>	Diptera (Agromyzidae)	Herbivore
83	<i>Phytomyza lateralis (cichorii)</i>	Diptera (Agromyzidae)	Herbivore
84	<i>Picris echioides</i>	Asterales (Asteraceae)	Plant
85	<i>Plantago lanceolata</i>	Lamiales (Plantaginaceae)	Plant
86	<i>Platycheirus scutatus</i>	Diptera (Syrphidae)	Natural enemy (predator)
87	<i>Poa pratensis</i>	Poales (Poaceae)	Plant

88	<i>Poa trivialis</i>	Poales (Poaceae)	Plant
89	<i>Poaceae</i>	Poales (Poaceae)	Plant
90	<i>Praon volucre</i>	Hymenoptera (Braconidae)	Natural enemy (parasitoid)
91	<i>Psilothrix viridicoeruleus</i>	Coleoptera (Dasytidae)	Herbivore
92	<i>Psyllobora vigintiduopunctata</i>	Coleoptera (Coccinellidae)	Herbivore
93	<i>Rhagonycha fulva</i>	Coleoptera (Cantharidae)	Natural enemy (predator)
94	<i>Rubus ulmifolius</i>	Rosales (Rosaceae)	Plant
95	<i>Rumex crispus</i>	Polygonales (Polygonaceae)	Plant
96	<i>Runcinia grammica</i>	Araneae (Thomisidae)	Omnivore
97	<i>Senecio vulgaris</i>	Asterales (Asteraceae)	Plant
98	<i>Sonchus asper</i>	Asterales (Asteraceae)	Plant
99	<i>Sonchus oleraceus</i>	Asterales (Asteraceae)	Plant
100	<i>Sphaerophoria sp.</i>	Diptera (Syrphidae)	Plant
101	<i>Stictopleurus punctatonervosus</i>	Hemiptera (Rhopalidae)	Omnivore
102	<i>Taraxacum officinale</i>	Asterales (Asteraceae)	Plant
103	<i>Cephalodo bifasciata</i>	Hymenoptera (Tenthredinidae)	Herbivore
104	<i>Tephritidae</i>	Diptera (Tephritidae)	Herbivore
105	<i>Tephritis cometa</i>	Diptera (Tephritidae)	Herbivore
106	<i>Tephritis formosa</i>	Diptera (Tephritidae)	Herbivore
107	<i>Tephritis sp.</i>	Diptera (Tephritidae)	Herbivore
108	<i>Tephritis vespertina</i>	Diptera (Tephritidae)	Herbivore
109	<i>Tortricidae</i>	Lepidoptera (Tortricidae)	Herbivore
110	<i>Trombidiidae</i>	Acariformes (Trombidiidae)	Natural enemy (Parasit)
111	<i>Tythaspis sedecimpunctata</i>	Coleoptera (Coccinellidae)	Omnivore
112	<i>Uroleucon sonchi</i>	Hemiptera (Aphididae)	Herbivore
113	<i>Verbascum blattaria</i>	Scrophulariales (Scrophulariaceae)	Plant
114	<i>Veronica persica</i>	Lamiales (Plantaginaceae)	Plant
115	<i>Vicia sativa</i>	Fabales (Fabaceae)	Plant
116	<i>Viola arvensis</i>	Violales (Violaceae)	Plant

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Table 4: List of interactions included in the sub-network (Figure 2)

Interaction	Resource	Consumer	Occurrence frequency
1	<i>Sonchus asper</i>	<i>Agromyzidae</i>	20
2	<i>Sonchus asper</i>	<i>Campiglossa producta</i>	13
3	<i>Sonchus asper</i>	<i>Ensina sonchi</i>	9
4	<i>Sonchus asper</i>	<i>Hyperomyzus lactucae</i>	17
5	<i>Sonchus asper</i>	<i>Liorhyssus hyalinus</i>	34
6	<i>Sonchus asper</i>	<i>Liriomyza sonchi</i>	14
7	<i>Sonchus asper</i>	<i>Lixus punctiventris</i>	2
8	<i>Sonchus asper</i>	<i>Ophiomyia cunctata (beckeri)</i>	8
9	<i>Sonchus asper</i>	<i>Philaenus spumarius</i>	9
10	<i>Sonchus asper</i>	<i>Phytomyza horticola (syngenesiae)</i>	15
11	<i>Sonchus asper</i>	<i>Psilothrix viridicoeruleus</i>	9
12	<i>Sonchus asper</i>	<i>Tephritis formosa</i>	7
13	<i>Sonchus asper</i>	<i>Tephritis sp</i>	11
14	<i>Sonchus oleraceus</i>	<i>Agapanthia cardui</i>	15
15	<i>Sonchus oleraceus</i>	<i>Agapanthia pannonica</i>	50
16	<i>Sonchus oleraceus</i>	<i>Agromyzidae</i>	52
17	<i>Sonchus oleraceus</i>	<i>Aphididae</i>	100
18	<i>Sonchus oleraceus</i>	<i>Aphis craccivora</i>	50
19	<i>Sonchus oleraceus</i>	<i>Aphis fabae</i>	71
20	<i>Sonchus oleraceus</i>	<i>Brassicogethes aeneus</i>	21
21	<i>Sonchus oleraceus</i>	<i>Campiglossa producta</i>	60
22	<i>Sonchus oleraceus</i>	<i>Cicadella viridis</i>	100
23	<i>Sonchus oleraceus</i>	<i>Closterotomus norvegicus</i>	100
24	<i>Sonchus oleraceus</i>	<i>Cnephasia stephensiana</i>	33
25	<i>Sonchus oleraceus</i>	<i>Curculionidae</i>	12
26	<i>Sonchus oleraceus</i>	<i>Cynipidae</i>	50
27	<i>Sonchus oleraceus</i>	<i>Dasytes tristiculus</i>	8
28	<i>Sonchus oleraceus</i>	<i>Dolycoris baccarum</i>	50
29	<i>Sonchus oleraceus</i>	<i>Ensina sonchi</i>	90
30	<i>Sonchus oleraceus</i>	<i>Hecatera dysodea</i>	100
31	<i>Sonchus oleraceus</i>	<i>Hyalopterus pruni</i>	100
32	<i>Sonchus oleraceus</i>	<i>Hyperomyzus lactucae</i>	69
33	<i>Sonchus oleraceus</i>	<i>Lepidargyrus ancorifer</i>	22
34	<i>Sonchus oleraceus</i>	<i>Liorhyssus hyalinus</i>	50
35	<i>Sonchus oleraceus</i>	<i>Liriomyza sonchi</i>	79
36	<i>Sonchus oleraceus</i>	<i>Lixus punctiventris</i>	96
37	<i>Sonchus oleraceus</i>	<i>Lygaeus equestris</i>	11
38	<i>Sonchus oleraceus</i>	<i>Macrosiphum rosae</i>	100
39	<i>Sonchus oleraceus</i>	<i>Miridae</i>	38

40	<i>Sonchus oleraceus</i>	<i>Nezara viridula</i>	33
41	<i>Sonchus oleraceus</i>	<i>Nysius cymoides</i>	12
42	<i>Sonchus oleraceus</i>	<i>Oedemera crassipes</i>	100
43	<i>Sonchus oleraceus</i>	<i>Oedemera flavipes</i>	33
44	<i>Sonchus oleraceus</i>	<i>Ophiomyia cunctata (beckeri)</i>	88
45	<i>Sonchus oleraceus</i>	<i>Orius sp</i>	50
46	<i>Sonchus oleraceus</i>	<i>Philaenus sp</i>	25
47	<i>Sonchus oleraceus</i>	<i>Philaenus spumarius</i>	40
48	<i>Sonchus oleraceus</i>	<i>Phytomyza horticola (syngenesiae)</i>	63
49	<i>Sonchus oleraceus</i>	<i>Phytomyza lateralis (cichorii)</i>	50
50	<i>Sonchus oleraceus</i>	<i>Psilothrix viridicoeruleus</i>	18
51	<i>Sonchus oleraceus</i>	<i>Stictopleurus punctatonervosus</i>	57
52	<i>Sonchus oleraceus</i>	<i>Cephalado bifasciata</i>	100
53	<i>Sonchus oleraceus</i>	<i>Tephritidae</i>	100
54	<i>Sonchus oleraceus</i>	<i>Tephritis cometa</i>	100
55	<i>Sonchus oleraceus</i>	<i>Tephritis formosa</i>	89
56	<i>Sonchus oleraceus</i>	<i>Tephritis sp</i>	48
57	<i>Sonchus oleraceus</i>	<i>Tephritis vespertina</i>	100
58	<i>Sonchus oleraceus</i>	<i>Tortricidae</i>	100
59	<i>Sonchus oleraceus</i>	<i>Uroleucon sonchi</i>	84
60	<i>Sonchus oleraceus</i>	<i>Coreus marginatus</i>	60
61	<i>Poa pratensis</i>	<i>Philaenus spumarius</i>	7
62	<i>Cirsium arvense</i>	<i>Aphis fabae</i>	14
63	<i>Cirsium arvense</i>	<i>Brassicogethes aeneus</i>	54
64	<i>Cirsium arvense</i>	<i>Oedemera flavipes</i>	33
65	<i>Cirsium arvense</i>	<i>Phytomyza horticola (syngenesiae)</i>	2
66	<i>Poaceae</i>	<i>Philaenus spumarius</i>	1
67	<i>Poaceae</i>	<i>Uroleucon sonchi</i>	1
68	<i>Picris echinoides</i>	<i>Agromyzidae</i>	1
69	<i>Picris echinoides</i>	<i>Brassicogethes aeneus</i>	7
70	<i>Picris echinoides</i>	<i>Hyperomyzus lactucae</i>	4
71	<i>Picris echinoides</i>	<i>Lepidargyrus ancorifer</i>	11
72	<i>Picris echinoides</i>	<i>Liorhyssus hyalinus</i>	3
73	<i>Picris echinoides</i>	<i>Nezara viridula</i>	17
74	<i>Picris echinoides</i>	<i>Philaenus spumarius</i>	9
75	<i>Picris echinoides</i>	<i>Phytomyza horticola (syngenesiae)</i>	15
76	<i>Picris echinoides</i>	<i>Phytomyza lateralis (cichorii)</i>	33
77	<i>Picris echinoides</i>	<i>Tephritis sp</i>	7
78	<i>Picris echinoides</i>	<i>Uroleucon sonchi</i>	1
79	<i>Lolium sp</i>	<i>Coreus marginatus</i>	20
80	<i>Veronica persica</i>	<i>Philaenus sp</i>	25
81	<i>Euphorbia helioscopia</i>	<i>Philaenus spumarius</i>	1
82	<i>Dicot unknown</i>	<i>Curculionidae</i>	12
83	<i>Dicot unknown</i>	<i>Philaenus sp</i>	25

84	<i>Lactuca serriola</i>	<i>Agromyzidae</i>	23
85	<i>Lactuca serriola</i>	<i>Aphis craccivora</i>	50
86	<i>Lactuca serriola</i>	<i>Hyperomyzus lactucae</i>	6
87	<i>Lactuca serriola</i>	<i>Liorhyssus hyalinus</i>	3
88	<i>Lactuca serriola</i>	<i>Lixus punctiventris</i>	2
89	<i>Lactuca serriola</i>	<i>Ophiomyia cunctata (beckeri)</i>	4
90	<i>Lactuca serriola</i>	<i>Philaenus spumarius</i>	1
91	<i>Lactuca serriola</i>	<i>Phytomyza horticola (syngenesiae)</i>	4
92	<i>Lactuca serriola</i>	<i>Phytomyza lateralis (cichorii)</i>	17
93	<i>Lactuca serriola</i>	<i>Uroleucon sonchi</i>	5
94	<i>Senecio vulgaris</i>	<i>Nezara viridula</i>	17
95	<i>Carduus pycnocephalus</i>	<i>Agapanthia cardui</i>	62
96	<i>Carduus pycnocephalus</i>	<i>Agromyzidae</i>	1
97	<i>Carduus pycnocephalus</i>	<i>Curculionidae</i>	76
98	<i>Carduus pycnocephalus</i>	<i>Cynipidae</i>	50
99	<i>Carduus pycnocephalus</i>	<i>Lepidargyrus ancorifer</i>	44
100	<i>Carduus pycnocephalus</i>	<i>Liorhyssus hyalinus</i>	3
101	<i>Carduus pycnocephalus</i>	<i>Miridae</i>	29
102	<i>Carduus pycnocephalus</i>	<i>Nezara viridula</i>	17
103	<i>Carduus pycnocephalus</i>	<i>Philaenus spumarius</i>	6
104	<i>Hordeum murinum</i>	<i>Philaenus spumarius</i>	1
105	<i>Cerastium glomeratum</i>	<i>Philaenus spumarius</i>	1
106	<i>Viola arvensis</i>	<i>Philaenus spumarius</i>	1
107	<i>Lathyrus sp</i>	<i>Philaenus spumarius</i>	4
108	<i>Geranium sp</i>	<i>Philaenus spumarius</i>	3
109	<i>Vicia sativa</i>	<i>Lygaeus equestris</i>	11
110	<i>Vicia sativa</i>	<i>Miridae</i>	8
111	<i>Vicia sativa</i>	<i>Philaenus spumarius</i>	4
112	<i>Anthemis altissima</i>	<i>Oedemera flavipes</i>	33
113	<i>Anthemis altissima</i>	<i>Psilothrix viridicoeruleus</i>	9
114	<i>Anthemis altissima</i>	<i>Tephritis sp</i>	4
115	<i>Avena sp</i>	<i>Miridae</i>	4
116	<i>Erigeron sp</i>	<i>Hyperomyzus lactucae</i>	2
117	<i>Erigeron sp</i>	<i>Psilothrix viridicoeruleus</i>	18
118	<i>Erigeron sp</i>	<i>Stictopleurus punctatonervosus</i>	14
119	<i>Papaver rhoeas</i>	<i>Brassicogethes aeneus</i>	4
120	<i>Papaver rhoeas</i>	<i>Miridae</i>	4
121	<i>Taraxacum officinale</i>	<i>Campiglossa producta</i>	13
122	<i>Taraxacum officinale</i>	<i>Phytomyza horticola (syngenesiae)</i>	2
123	<i>Taraxacum officinale</i>	<i>Stictopleurus punctatonervosus</i>	14
124	<i>Crepis vesicaria</i>	<i>Aphis fabae</i>	14
125	<i>Crepis vesicaria</i>	<i>Campiglossa producta</i>	13
126	<i>Crepis vesicaria</i>	<i>Tephritis formosa</i>	3
127	<i>Crepis vesicaria</i>	<i>Tephritis sp</i>	26



128	<i>Poa trivialis</i>	<i>Orius sp</i>	50
129	<i>Poa trivialis</i>	<i>Philaenus spumarius</i>	1
130	<i>Plantago lanceolata</i>	<i>Uroleucon sonchi</i>	2
131	<i>Cichorium intybus</i>	<i>Philaenus spumarius</i>	1
132	<i>Cichorium intybus</i>	<i>Uroleucon sonchi</i>	1
133	<i>Crepis foetida</i>	<i>Lepidargyrus ancorifer</i>	11
134	<i>Crepis foetida</i>	<i>Nysius cymoides</i>	12
135	<i>Knautia arvensis</i>	<i>Philaenus sp</i>	25
136	<i>Rumex crispus</i>	<i>Philaenus spumarius</i>	3
137	<i>Rubus ulmifolius</i>	<i>Philaenus spumarius</i>	1
138	<i>Geranium rotundifolium</i>	<i>Liorhyssus hyalinus</i>	3
139	<i>Geranium rotundifolium</i>	<i>Uroleucon sonchi</i>	6
140	<i>Hypericum perforatum</i>	<i>Coreus marginatus</i>	20
141	<i>Cirsium vulgare</i>	<i>Nysius cymoides</i>	12
142	<i>Lepidium draba</i>	<i>Nysius cymoides</i>	38
143	<i>Verbascum blattaria</i>	<i>Lepidargyrus ancorifer</i>	11
144	<i>Verbascum blattaria</i>	<i>Nysius cymoides</i>	25
145	<i>Medicago orbicularis</i>	<i>Dolycoris baccarum</i>	17
146	<i>Medicago orbicularis</i>	<i>Nezara viridula</i>	17
147	<i>Psilothrix viridicoeruleus</i>	<i>Neoscona adianta</i>	33
148	<i>Brassicogethes aeneus</i>	<i>Misumena vatia</i>	17
149	<i>Brassicogethes aeneus</i>	<i>Neoscona adianta</i>	33
150	<i>Brassicogethes aeneus</i>	<i>Runcinia grammica</i>	23
151	<i>Oedemera flavipes</i>	<i>Runcinia grammica</i>	8
152	<i>Ophiomyia cunctata (beckeri)</i>	<i>Braconidae</i>	12
153	<i>Phytomyza horticola (syngenesiae)</i>	<i>Bracon telengai</i>	33
154	<i>Phytomyza horticola (syngenesiae)</i>	<i>Dacnusa maculipes</i>	100
155	<i>Phytomyza horticola (syngenesiae)</i>	<i>Opiinae</i>	100
156	<i>Tephritidae</i>	<i>Bracon radialis</i>	67
157	<i>Tephritidae</i>	<i>Bracon sp</i>	28
158	<i>Tephritidae</i>	<i>Braconidae</i>	12
159	<i>Campiglossa producta</i>	<i>Bracon radialis</i>	33
160	<i>Campiglossa producta</i>	<i>Bracon sp</i>	6
161	<i>Campiglossa producta</i>	<i>Runcinia grammica</i>	8
162	<i>Ensina sonchi</i>	<i>Bracon sp</i>	22
163	<i>Ensina sonchi</i>	<i>Bracon telengai</i>	33
164	<i>Ensina sonchi</i>	<i>Braconidae</i>	75
165	<i>Ensina sonchi</i>	<i>Coreus marginatus</i>	40
166	<i>Ensina sonchi</i>	<i>Kochiura aulica</i>	17
167	<i>Ensina sonchi</i>	<i>Liorhyssus hyalinus</i>	3
168	<i>Ensina sonchi</i>	<i>Lygaeus equestris</i>	56
169	<i>Ensina sonchi</i>	<i>Nysius cymoides</i>	12
170	<i>Tephritis formosa</i>	<i>Bracon sp</i>	44
171	<i>Tephritis formosa</i>	<i>Bracon telengai</i>	33

172	<i>Tephritis formosa</i>	<i>Kochiura aulica</i>	17
173	<i>Tephritis formosa</i>	<i>Lygaeus equestris</i>	11
174	<i>Tephritis formosa</i>	<i>Miridae</i>	4
175	<i>Aphis fabae</i>	<i>Liorhyssus hyalinus</i>	3
176	<i>Aphis fabae</i>	<i>Lipolexis gracilis</i>	100
177	<i>Aphis fabae</i>	<i>Lygaeus equestris</i>	11
178	<i>Aphis fabae</i>	<i>Praon volucre</i>	9
179	<i>Hyalopterus pruni</i>	<i>Aphidius sonchi</i>	4
180	<i>Hyperomyzus lactucae</i>	<i>Alloxysta consobrina</i>	50
181	<i>Hyperomyzus lactucae</i>	<i>Aphidius sonchi</i>	83
182	<i>Hyperomyzus lactucae</i>	<i>Leptophyes punctatissima</i>	20
183	<i>Hyperomyzus lactucae</i>	<i>Miridae</i>	8
184	<i>Hyperomyzus lactucae</i>	<i>Praon volucre</i>	86
185	<i>Hyperomyzus lactucae</i>	<i>Sphaerophoria sp</i>	11
186	<i>Hyperomyzus lactucae</i>	<i>Stictopleurus punctatonervosus</i>	14
187	<i>Hyperomyzus lactucae</i>	<i>Trombidiidae</i>	50
188	<i>Hyperomyzus lactucae</i>	<i>Tytthaspis sedecimpunctata</i>	14
189	<i>Aphididae</i>	<i>Alloxysta consobrina</i>	50
190	<i>Aphididae</i>	<i>Platycheirus scutatus</i>	100
191	<i>Uroleucon sonchi</i>	<i>Aphidius ervi</i>	100
192	<i>Uroleucon sonchi</i>	<i>Coccinella septempunctata</i>	14
193	<i>Uroleucon sonchi</i>	<i>Ephedrus sp</i>	80
194	<i>Uroleucon sonchi</i>	<i>Episyrphus balteatus</i>	6
195	<i>Uroleucon sonchi</i>	<i>Heliophanus sp</i>	50
196	<i>Uroleucon sonchi</i>	<i>Praon volucre</i>	5
197	<i>Uroleucon sonchi</i>	<i>Psyllobora vigintiduopunctata</i>	9
198	<i>Uroleucon sonchi</i>	<i>Rhagonycha fulva</i>	22
199	<i>Philaenus spumarius</i>	<i>Heliophanus cupreus</i>	33
200	<i>Miridae</i>	<i>Ephedrus sp</i>	10
201	<i>Miridae</i>	<i>Runcinia grammica</i>	8
202	<i>Lepidargyrus ancorifer</i>	<i>Runcinia grammica</i>	8
203	<i>Liorhyssus hyalinus</i>	<i>Kochiura aulica</i>	17
204	<i>Hecatera dysodea</i>	<i>Microplitis sp</i>	100
205	<i>Tortricidae</i>	<i>Diadegma erucator</i>	75
206	<i>Hypochoeris radicata</i>	<i>Agromyzidae</i>	1
207	<i>Psyllobora vigintiduopunctata</i>	<i>Misumena vatia</i>	17
208	<i>Episyrphus balteatus</i>	<i>Misumena vatia</i>	17
209	<i>Platycheirus scutatus</i>	<i>Diplazon tibiatorius</i>	100
210	<i>Sphaerophoria sp</i>	<i>Lygaeus equestris</i>	11
211	<i>Sphaerophoria sp</i>	<i>Misumena vatia</i>	50
212	<i>Sphaerophoria sp</i>	<i>Neoscona adianta</i>	67
213	<i>Sphaerophoria sp</i>	<i>Runcinia grammica</i>	15

## Supplementary Figures

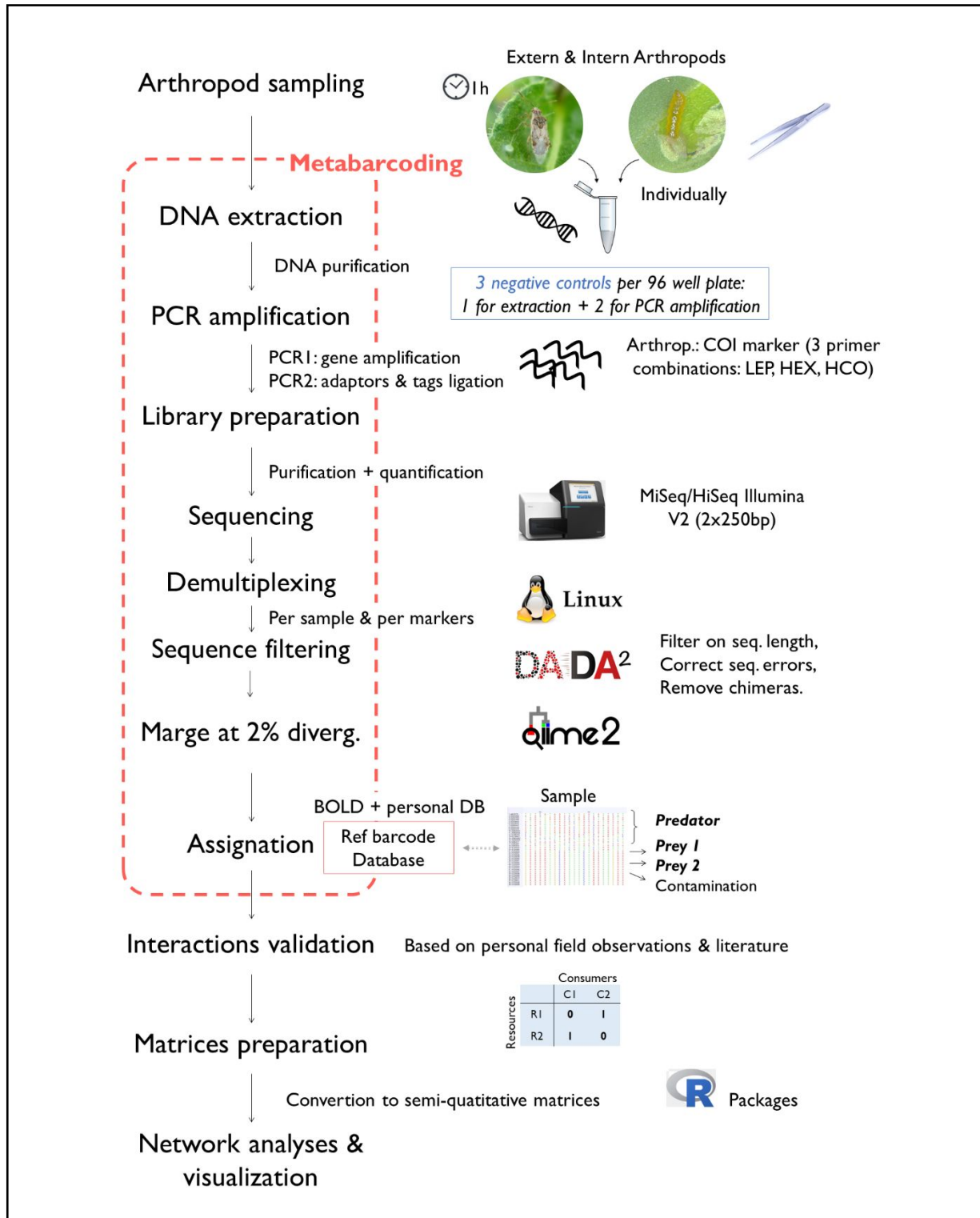


Figure S1: Overview of the entire process for the reconstruction of interactions between plants, herbivores and natural enemies.

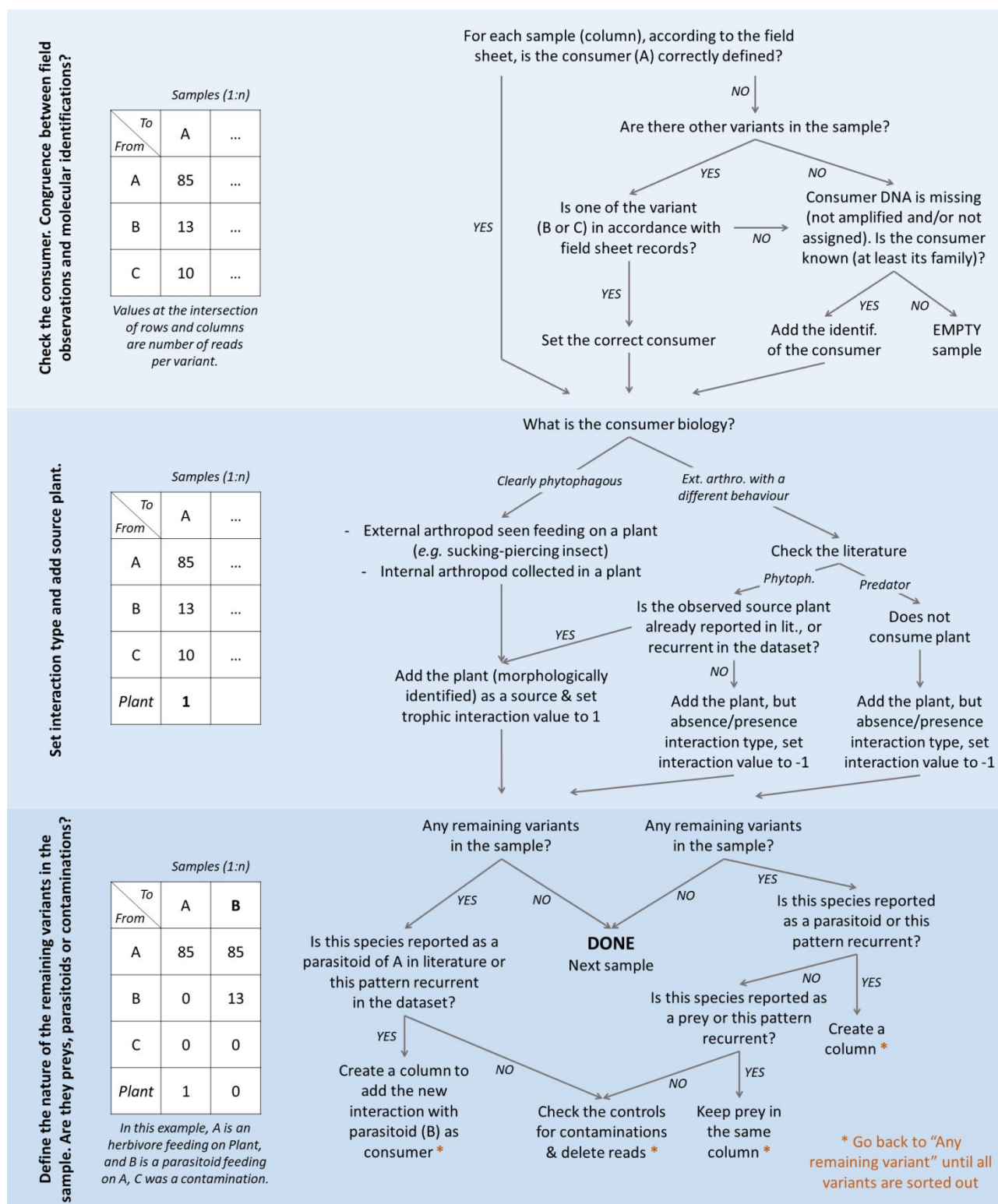


Figure S2: Flow diagram presenting the process followed for the transformation of raw data from sequencing into an adjacency matrix.

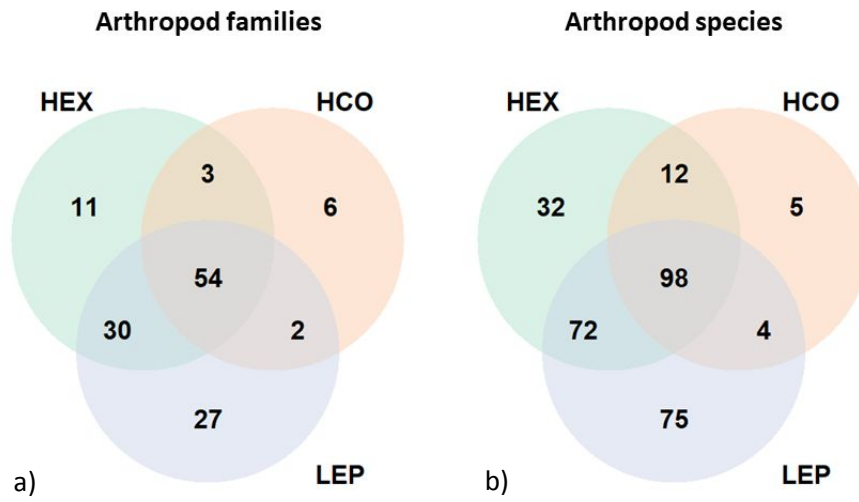
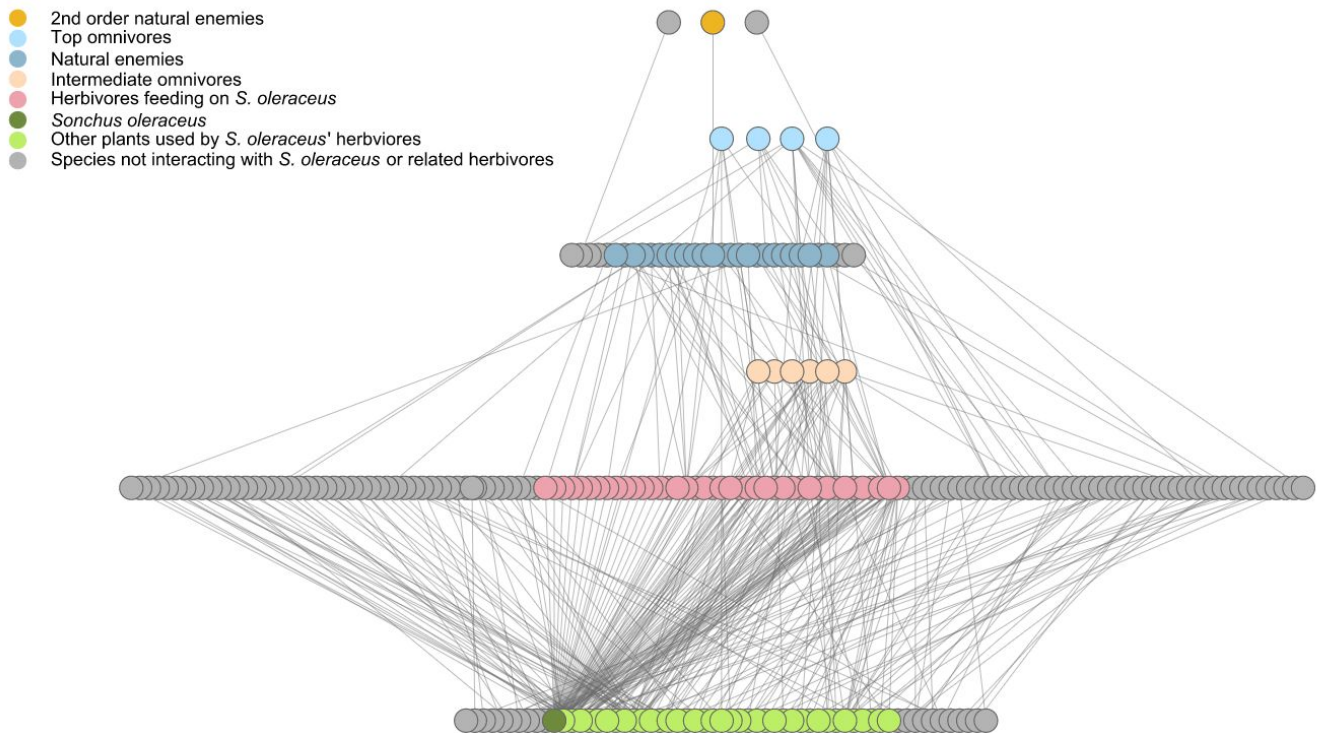


Figure S3: Venn diagram of taxonomic coverage obtained with the three COI markers used in this study, represented through a) arthropod family diversity and b) arthropod species diversity.



Figure S4: Taxonomic abundance of a) plants and b) arthropods in the dataset after data validation. This diagram allows visualizing the hierarchical organisation of taxonomic affiliation for each species, considering species abundance. E.g. *Tephritis Formosa* and *Ensina sonchi* (*Tephritidae*: *Diptera*) were two arthropod species very frequently sampled during this campaign (Spring 2018) representing 13% and 15% of samples collected, respectively.





*Figure S5 : Meta-network reconstructed through intense sampling in France during spring 2018. Colored nodes refer to subnetwork centred on *S. oleraceus* related interactions. The meta-network is composed of 241 nodes and 350 links, the subnetwork is composed of 116 nodes and 213 links.*

## References cited in supplementary Text 1

- Alberdi A, Aizpurua O, Gilbert MTP, Bohmann K (2018) Scrutinizing key steps for reliable metabarcoding of environmental samples. *Methods in Ecology and Evolution* 9:134–147. <https://doi.org/10.1111/2041-210X.12849>
- Deagle BE, Thomas AC, McInnes JC, et al (2019) Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? *Molecular Ecology* 28:391–406. <https://doi.org/10.1111/mec.14734>
- R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Taberlet P, Bonin A, Coissac E, Zinger L (2018) *Environmental DNA: For Biodiversity Research and Monitoring*. Oxford University Press