

1 **Agricultural pressures impair trophic link between aquatic microorganisms**
2 **and invertebrates**

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19 **Main finding:** *Under laboratory and field conditions, we uncover how invertebrate feeding*
20 *on microbial decomposition are two intertwined ecosystem processes that present a currently*
21 *underappreciated trophic link vulnerable to agricultural chemicals.*

22

23

24 **Abstract:** Decadal declines in aquatic ecosystem health prompted monitoring efforts and
25 studies on effects of human practices on aquatic biodiversity, yet a consideration of ecological
26 processes and trophic linkages is increasingly required to develop an in-depth understanding of
27 aquatic food webs and its vulnerability to human activities. Here, we test in laboratory
28 incubations using natural organic matter whether agricultural practices have an effect on two
29 interacting ecological processes (i.e., decomposition and invertebrate growth) as the relevant
30 temporal components of the trophic linkage between aquatic microbial communities and
31 aquatic invertebrates. We further assess whether these altered trophic interactions are visible on
32 ecologically relevant scales. We observed clear patterns in agricultural constraints on microbial
33 decomposition, which coincided with reduced invertebrate growth and an unexpected increase
34 in invertebrate consumption of organic matter. Similar differences in invertebrate length
35 depending on land use were observed in our field survey, thereby providing important clues on
36 the relevance and vulnerability of interdependent processes that can serve to improve future
37 forays in monitoring ecosystem health.

38

39 **Keywords:**

40 *Asellus aquaticus, decomposition, DECOTAB, ecosystem functioning, organic matter,*
41 *microorganisms*

42 **Introduction**

43 Globally, anthropogenic activities pose significant threats to aquatic ecosystems
44 (Strayer & Dudgeon, 2010; Vörösmarty et al., 2010; Strayer, 2010a). Resulting declines in
45 ecosystem health have fueled monitoring activities, which are primarily aimed at quantifying
46 biodiversity and ecosystem processes to allow for diagnosing perturbations and safeguarding
47 the natural environment. A key ecosystem process proven vulnerable to such stressors is the
48 degradation of dead organic matter (OM), such as decaying plant litter, that serves as food for
49 aquatic microorganisms and invertebrates (Webster & Benfield, 1986; Wallace & Webster,
50 1996; Bundschuh & McKie, 2016). This process relies on a complex interplay between
51 microorganisms and invertebrates where microbial conditioning of OM enhances nutritional
52 value for invertebrates, while consumption of OM by invertebrates renders a diverse size range
53 of dissolved and particulate OM that supports microorganism as well as filter-and deposit
54 feeding invertebrates (Barlöcher, 1985; Graça et al., 1993; Graça, 2001; Wright & Covich 2005;
55 Danger et al., 2012; Danger et al., 2012; Arce Funck et al., 2015; Vonk et al., 2016; Bundschuh
56 & McKie, 2016). While most studies and monitoring efforts focus on diversity measures such
57 as species abundances within a single trophic level, an explicit consideration of the complexity
58 of interactions is now crucial to develop an in-depth understanding of aquatic food webs and
59 its vulnerability to human activities (Hines et al., 2016; Schrama et al., 2017; Seibold et al.,
60 2018; Bruder et al., 2019).

61 Agricultural practices are a primary source of pollution to aquatic systems. This is due to
62 the use of chemicals such as pesticides and fertilizer that run-off to adjacent aquatic systems
63 where they can have direct- or indirect toxic effects in the water column or associate with OM
64 accumulating in their sediments (e.g., Knezovich et al., 1987; Vijver et al., 2017). To date,
65 studies aimed at assessing effects of agricultural practices mostly focussed on direct effects
66 (e.g., toxicity) to aquatic invertebrates, while studies focused on indirect effects of chemicals

67 on invertebrates relied predominantly on feeding preference experiments and singled-out
68 chemicals that are often applied to the overlying water (e.g. Feckler et al., 2016; Vijver et
69 al., 2017; Rossi et al., 2018; Barmantlo et al., 2018, 2019). This approach prevents the
70 distinction between direct and indirect effects on microbially-mediated trophic linkages that
71 are caused by chemical deposition and sorption of chemical to OM on temporal scales that are
72 relevant to ecosystem processes.

73 Since various chemicals have been observed to affect microbial community composition
74 and diversity (Gardeström et al., 2016; Tlili et al., 2016; Hunting et al., 2017a, Fasching et al.,
75 2019), alterations in microbial diversity can be expected to affect the overall rate of microbial
76 decomposition. This is relevant since the process of microbial decomposition, in contrast to
77 microbial diversity, provides a more reliable reflection of OM-conditioning and available
78 microbial biomass, and therewith the resources available for invertebrates, for the time period
79 (seasons to years) that is required for OM to decompose and invertebrates to develop (e.g.,
80 Hines et al., 2016). However, as with invertebrates, the vast majority of studies on microbial
81 communities rely on single time point characterization of microbial diversity or exposure to
82 manipulated concentrations of model chemical compounds. It thus remains uncertain whether
83 complex mixtures of organic particles and pollutants currently present in the environment
84 translate to altered microbial processes, and whether changes in the temporal attributes of
85 microbial processing of OM affect invertebrate performance.

86 This study tests whether the chemical complexity associated with agricultural practices
87 affects microbial processing of OM and if this translates to aquatic invertebrates that rely on
88 microbial biomass and microbial conditioning of OM. To this end, we assessed the effects of
89 agricultural practices on 1) the contribution of microbial communities on decomposition of OM
90 derived from an agricultural area, and 2) how changes in OM microbial decomposition affect
91 the growth of the aquatic invertebrate *Asellus aquaticus* under controlled laboratory conditions.

92 Finally, we surveyed *A. aquaticus*' body length in relation to agricultural practices within the
93 same agricultural area.

94

95 **Material and methods**

96 Experimental approach and research setting: We performed both a laboratory feeding
97 experiment and a field survey to determine the effect of agricultural practices on microbial OM
98 decomposition and invertebrate growth. In both components of the study, we exposed *Asellus*
99 *aquaticus* individuals to OM-rich sediments originating from ditches adjacent to the three key
100 types of land use present in an agricultural area, 'Bollenstreek', the Netherlands (Lat:
101 52°15'55.66", Long: 4°28'27.94"), that have well-documented effects on microbial community
102 structure and decomposition (Hunting et al., 2016; 2017a). Land uses selected were a dune
103 nature reserve, agricultural sites growing flower bulb (hyacinths, lilies, tulips and daffodils) and
104 permanent grassland. The agricultural area is intensively used to grow flower bulbs, which are
105 treated with pesticides and nutrients between February to November. The ditches are adjacent
106 to the flower bulb fields, which results in the transfer of pesticides and nutrients from the fields
107 into the ditches. The grassland pastures are used for grazing by livestock. Natural sandy dunes
108 can be found to the north and north-west of the area at approximately 200m from the ditches.
109 The water bodies located in this area are hydrologically isolated from the agricultural area
110 because of a natural elevation gradient (Ieromina et al., 2015). All water bodies are
111 characterized by a wide variety of aquatic invertebrates (Ieromina et al., 2015; Hunting et al.,
112 2016).

113

114 Feeding experiment: To assess whether changes in microbial decomposition of OM affect the
115 growth of aquatic invertebrates, we performed a laboratory feeding assay where we placed

116 *Asellus aquaticus* (being a common detritivore in many freshwater habitats) on a diet of OM
117 collected from ditches adjacent to either flower bulb fields, grasslands or pristine dune area. *A.*
118 *aquaticus* was chosen as a model organism as it is a highly abundant detritivore in aquatic ditch
119 systems. We prepared Decomposition and Consumption Tablets (DECOTABs) as standardized
120 OM substrate as described by Kampfraath et al. (2012) and Van der Lee (2020). In brief,
121 DECOTABs (ϕ 17 mm) were prepared from naturally-derived OM embedded in an agar matrix,
122 containing OM derived from sediments adjacent to flower bulb fields, grasslands or pristine
123 dune area. DECOTABs were prepared as explained in detail in Hunting et al., 2016. Individuals
124 of *A. aquaticus* used in the experiment were reared in the laboratory. Prior to the experiment,
125 an egg-carrying female was isolated and placed in Elendt M4 medium (OECD, 2004) on a small
126 layer (~1 cm) of fine quartz sand. In total, 36 neonates of *A. aquaticus* were collected directly
127 after hatching. As such, all collected neonates were from the same spawn which limits variation
128 in body length between individuals at the start of the experiment. Subsequently, neonates were
129 placed in 50 mL glass bottles individually, which were filled with 35 mL Elendt M4 medium.
130 One DECOTAB was added to each bottle. In total, 36 bottles containing one *A. aquaticus*
131 neonate and one DECOTAB each ($n = 12$ per land use type) were used in the experiment.
132 Additionally, per treatment, three control bottles containing one DECOTAB and without
133 invertebrates were used to assess substrate dependent microbial decomposition rates. The
134 experiment was conducted at a constant temperature of 18 °C and a light-dark regime of 16:8
135 hours for the duration of 42 days. Elendt M4 medium was replaced with freshly prepared
136 medium every seven days. Physicochemical parameters (pH, dissolved oxygen, electrical
137 conductivity, and temperature) were measured weekly using a HACH HQ40D electronic multi
138 meter to ensure they were comparable between treatments throughout the experiment (data not
139 shown). From $t=14$ onwards, *A. aquaticus* individuals were photographed every seven days
140 using an eScope DP-M17 USB-microscope camera to monitor growth rates. Subsequently, *A.*

141 *aquaticus* body length was quantified by measuring from cephalothorax to pleotelson from a
142 dorsal perspective using ImageJ (v1.47). Potential effects of DECOTABs per land use, time (in
143 days), and their respective interaction on *A. aquaticus*' body length were analyzed using a linear
144 mixed effect model in R (R Core Team, 2019), with the individual animals added as random
145 variable (to account for the repeated measures design). To meet the assumption of normality of
146 the model residuals and the random variable, body length data was log₁₀ transformed.
147 Furthermore, analysis of mortality data was performed by means of a logistic regression. After
148 termination of the experiment (42 days after inoculation), remaining DECOTAB material was
149 collected, dried at 60 °C for 48 hours and weighed on a balance (BP210S, Sartorius AG). In
150 addition, DECOTABs which were only exposed to substrate-dependent microbial
151 decomposition were dried and weighed following the same protocol. In order to assess initial
152 DECOTAB weight, ten unused DECOTABs per treatment were dried and weighed following
153 the same. Thereafter, both microbial decomposition and isopod consumption of OM was
154 analyzed using a one way-ANOVA with a Tukey HSD post-hoc test.

155

156 Field survey: To determine the effect of agricultural practices on aquatic invertebrate growth
157 in the field, we collected *A. aquaticus* individuals from the top sediment layer by grab sampling
158 using a 500µm dipping net at the same sites where the organic material for the lab experiment
159 was retrieved. All specimens were collected on the same day in June 2016. At each of the three
160 sites corresponding with the three types of land use (bulb, grassland, and pristine dune area),
161 roughly 20 *A. aquaticus* individuals were collected, with a total of 57 individuals. Sexes were
162 determined by examining the morphological properties as described by Bertin & Cézilly (2003).
163 Individual *A. aquaticus* collected from the field were photographed and measured as stated
164 earlier. An analysis of the effect of land use adjacent to the habitat on the body length of field-
165 collected *A. aquaticus* was conducted using a one-way ANOVA model with length of each

166 specimen as a dependent variable and land use as a predictor. Because *A. aquaticus* sexes
167 typically differ in body length, we carried out separate analyses for both sexes.

168

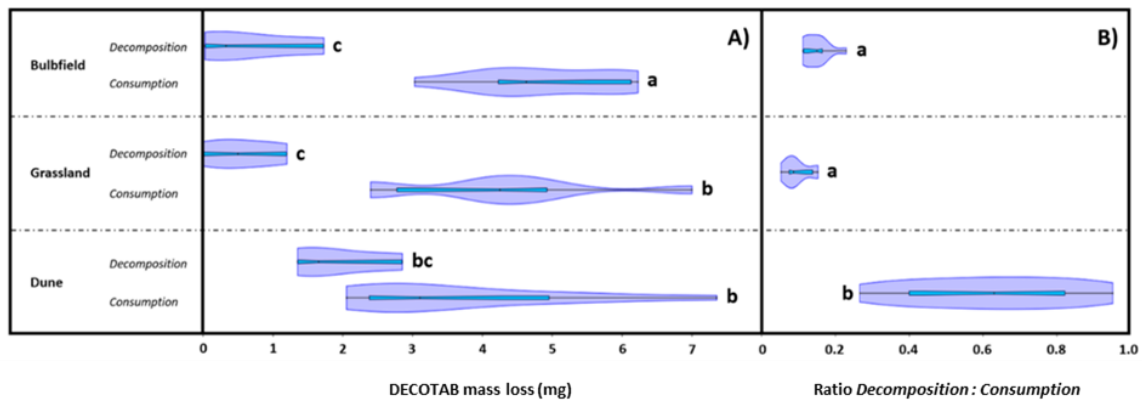
169 Agricultural practices in the study area have well documented effects on microbial
170 decomposition and community composition (Hunting et al., 2016; 2017a). A full assessment of
171 microbial parameters was therefore considered to be superfluous and beyond the scope of this
172 study. To confirm that agricultural practices in the study area differentially affected microbial
173 communities during this study period, we measured carbon utilization profiles of sediment
174 bacteria as a proxy of microbial functional (metabolic) diversity using Ecoplates (Biolog).
175 Ecoplates are comprised of ecologically relevant, structurally diverse compounds, yet do not
176 include e.g., recalcitrant substrates nor specific substrates typical of the soils used in this study.
177 It is therefore impossible to directly relate substrate utilization profiles to the actual functioning
178 of the soil microbial communities. Nonetheless, the number of substrates used can serve as a
179 proxy of the metabolic diversity of the microbial community (Garland, 1999). To this end,
180 microbial communities were sampled from sediments adjacent to dunes, grasslands, and bulb
181 fields (6 replicates per treatment). One mL of sediment was diluted 50 times with demineralized
182 water and vortexed. Mineral substrate was allowed to settle and subsequently distributed over
183 well plates (Biolog, Ecoplate) within 2 hours after sampling. Plates were incubated for 96 h at
184 18°C and absorbance was measured using a standard plate reader. Carbon utilization profiles
185 were analyzed using principal component analysis and a Jaccard-based one-way analysis of
186 similarity (ANOSIM).

187

188 **Results**

189 After 42 days of incubation, *Asellus aquaticus* contributed significantly (ANOVA, $F =$
190 56.8, $p < 0.001$) more to DECOTAB mass loss, when compared to DECOTAB exposed solely

191 to microbial biofilms (Fig 1A). A shift in relative contributions to OM degradation becomes
192 evident when plotting the ratio of microbial decomposition and isopod-associated OM
193 consumption (Fig 1B), illustrating a shift towards a higher contribution of microbial
194 decomposition to OM degradation. Isopods contributed to 37.8% of biomass loss of dune-
195 derived OM, whereas they contributed to 85.2% and 90.3% of biomass loss in bulb field- and
196 grassland-derived OM, respectively. We observed major differences between the ratios of
197 microbial decomposition and isopod-associated degradation of bulb field- and grassland
198 derived OM on the one hand and dune-derived OM on the other hand.
199

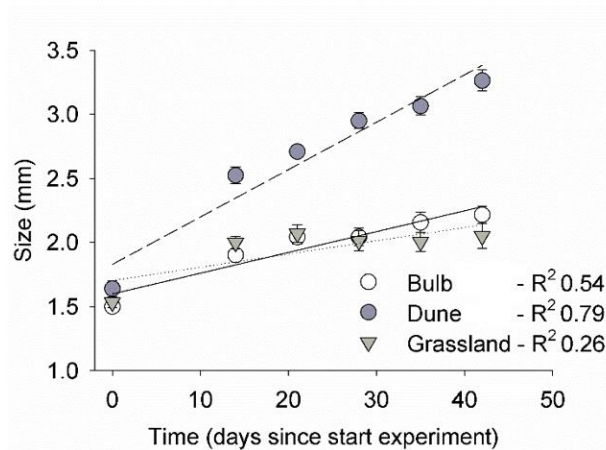


200
201 **Figure 1.** Organic matter mass loss after 42 days of microbial decomposition (\pm SE, $n = 3$ for dune, grass and
202 bulb) for each land use type (ANOVA; significance level $p = 0.05$) and *Asellus aquaticus* consumption (A; \pm SE,
203 $n = 12$ for dune, $n = 8$ for grass, and $n = 11$ for bulb; only surviving individuals are shown) and ratio between
204 microbial decomposition and isopod consumption of organic matter (B).
205

206 Over the course of 42 days, we observed that *A. aquaticus* feeding on OM derived from
207 the dune area had significantly higher growth rates compared to *A. aquaticus* that fed on
208 DECOTABs with grass- or bulb OM (TukeyHSD: $p_{\text{dune-bulb}} < 0.001$ and $p_{\text{dune-grass}} < 0.001$),
209 whereas no difference in growth rates was observed between *A. aquaticus* that fed on bulb-and
210 grass OM (TukeyHSD: $p = 0.377$; Fig. 2). In addition, *A. aquaticus* that fed on grassland-

211 derived OM had lower survival (66.7%) at the end of the experiment compared to juveniles that
212 fed on dune- (100%) and/or bulb (91.7%) derived OM (Chi-square, $p = 0.03$).

213



214

215 **Figure 2.** Body length (\pm SE, $n = 12$) of *Asellus aquaticus* during 42 days of incubation feeding on DECOTABs
216 inoculated with organic matter from ditches adjacent to bulb fields ($y = 1.69 + 0.011x$), pristine dune area (y
217 $=1.82+0.037x$) grassland ($y = 1.59 + 0.0163x$) (LME, significance level $p = 0.05$). On average, *A. aquaticus* which
218 fed on organic matter collected from ditches adjacent to pristine dune areas displayed a significantly higher growth
219 rate in comparison to those which fed on organic matter collected from ditches adjacent to bulb fields and
220 grasslands.

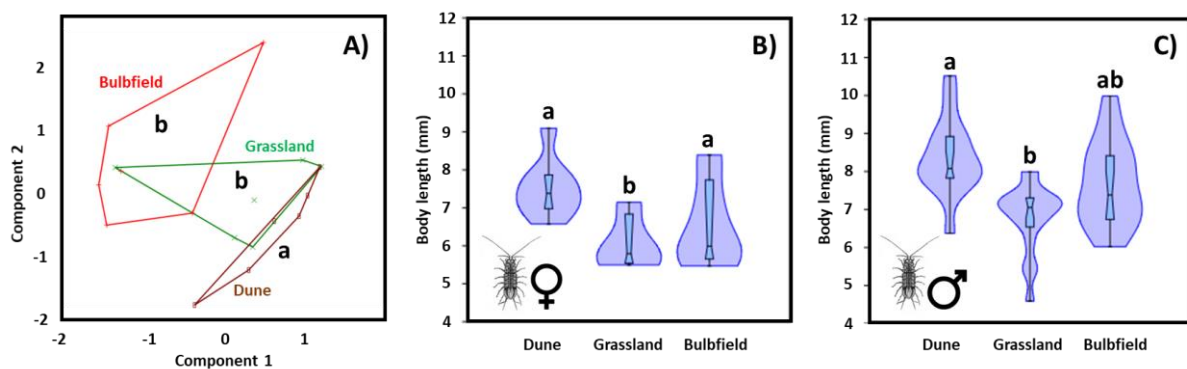
221

222 In our field survey, we observed differences in microbial carbon utilization profiles
223 depending on land use (Fig. 3A: ANOSIM; $p < 0.05$). This indicates differences in metabolic-
224 and functional diversity of microbial communities between land use types. Analysis of body
225 length of both sexes combined suggests an effect of land use, as individuals collected from the
226 dune area were larger than those collected from the bulb- and grassland area (LME, $p = 0.042$).
227 Males constituted the majority of the collected *A. aquaticus* individuals were (40 males vs 17
228 females). Male *A. aquaticus* were larger than female individuals (figure 3BC), which is
229 commonly observed for this species (Bertin & Cézilly, 2003). Body length of *A. aquaticus*
230 collected from the dune area were largest, and those from the grassland area were the smallest;

231 individuals from bulb fields were of intermediate length and did not appear to differ from
232 individuals from other land uses. This pattern was only observed in male individuals (Fig. 3B:
233 TukeyHSD: $p_{\text{dune vs grass}} < 0.001$, $p_{\text{grass vs bulb}} = 0.03$). Female individuals collected from grassland
234 ditches were smaller compared to individuals collected from ditches in dune areas (Fig. 3C:
235 TukeyHSD: $p_{\text{dune-grass}} = 0.049$).

236

237



238

239 **Figure 3.** Microbial carbon utilization profiles of microbial communities extracted from sediments collected from
240 ditches adjacent to dune-, bulb field- and grassland areas (A; $n = 6$; ANOSIM; $p < 0.05$) and average body length
241 (\pm SE, B: $n = 5-8$; C: $n = 14-18$; ANOVA + Tukey post-hoc; significance level $p = 0.05$) of female (B) and male
242 (C) *Asellus aquaticus* collected from ditches adjacent to a pristine dune area, grasslands and bulb fields.
243 Corresponding letters indicate statistical similarity.

244

245

246 **Discussion**

247 This study tested whether agricultural practices have an effect on two ecological
248 processes (i.e., decomposition and invertebrate growth) that represent relevant temporal
249 components of the trophic linkage between aquatic microbial communities and aquatic
250 invertebrates, and assessed whether potential alterations are visible on ecologically relevant
251 scales. We observed clear patterns in agricultural practices on microbial decomposition of OM
252 collected in the field, which cascaded to effects on higher trophic levels by reduced invertebrate
253 growth of both laboratory incubated and field-collected invertebrates. This carries implications
254 for a range of ecological settings and current assessments of ecosystem health.

255 While microbial communities in natural systems are often considered resilient to natural
256 disturbances and species loss due to a certain level of functional redundancy (e.g., Langenheder
257 et al., 2005), a wide array of chemical pressures (e.g., agricultural chemicals and nanoparticles)
258 have been shown to govern microbial community assembly and decomposition (Tlili et al.,
259 2016; Hunting et al., 2016; 2017a; Zhai et al. 2018;). Here, we also observed microbial
260 communities in the field to differ in their metabolic potential, and OM collected in the field to
261 differ in microbial decomposition depending on the type of agricultural practices. In addition
262 to direct toxic effects of chemicals, different organic practices result in differences in OM-
263 subsidy (e.g., different crops) as well as sorption of agricultural chemicals to OM, affecting
264 both quantity and quality of OM. This is relevant as microbial community assembly and
265 processes are frequently observed to be driven by quality of the available OM resources (e.g.,
266 Myers et al., 2001; Docherty et al., 2006; Strickland et al., 2009; Hunting et al., 2013). The
267 observed adverse effects of agricultural chemical on microbial decomposition of OM may be
268 relevant to a wide array of chemicals present in agricultural catchments, provided hydrophobic
269 fungicides can also inhibit fungal hyphomycete growth and microbial community composition
270 (Zubrod et al., 2015; Korschak et al., 2019).

271 In contrast to measures of microbial diversity, decomposition rates provide a more
272 reliable reflection of the available microbial biomass over extended periods of time. To assess
273 whether differences in microbial decomposition translate to differential performances in a
274 higher trophic level, growth rates of *Asellus aquaticus* were measured in relation to OM
275 decomposition. We observed that *A. aquaticus* performed better when feeding on OM derived
276 from dunes compared to bulb- and grassland-derived OM. This suggests invertebrate growth
277 follows similar patterns as microbial decomposition depending on type of land use, i.e., higher
278 growth rates with higher microbial decomposition rates. Dissimilarities in palatability and
279 nutritional value between the different OM sources and their associated microbial communities
280 can thus become visibly in invertebrate growth and size. While direct effects of chemicals and
281 their metabolites present in the OM on invertebrate performance cannot be excluded (Zubrod
282 et al., 2010), reduced growth rates have been causally linked to an absence of OM-associated
283 microorganisms, emphasizing the importance of microorganisms in the diet of invertebrate
284 shredders (Zhai et al. 2018). This corroborates the notion that colonization and conditioning of
285 OM by microorganisms plays an important role in invertebrate nutrition and invertebrate-
286 mediated consumption of OM (Graça et al., 1993; Graça, 2001; Wright & Covich 2005; Danger
287 et al., 2012; Hines et al., 2016), and suggests that observed growth reductions in response to
288 agricultural practices is due to a decrease in microbial biomass availability over the course of
289 the experiment. This seems to have prompted *A. aquaticus* to feed directly on OM rather than
290 microbial biomass, as we observed an unexpected increase in consumption of bulb- and
291 grassland-derived OM that apparently contained fewer essential nutrients such as fatty acids
292 and proteins to support growth. This points to a currently underappreciated compensatory
293 feeding mechanism in invertebrates in response to microbial perturbations.

294 Conventional monitoring efforts aimed at assessing ecosystem health generally relies
295 on single time point quantification of species diversity and abundance with a large emphasis on

296 invertebrates (Hunting et al., 2017b). Often, stressor-induced effects are not directly reflected
297 by species diversity and abundance alone, but also by characteristics related to species-specific
298 life-history, such as body length, that capture the long-term result of exposure to stress (Culp et
299 al., 2010; Hines et al., 2016). Here, agricultural practices were observed to have an effect on
300 two ecological processes (i.e., decomposition and invertebrate growth) that capture the temporal
301 complexity of the trophic linkage between aquatic microbial communities and aquatic
302 invertebrates. The observed adverse effects of agricultural stressors on the performance of OM-
303 associated microbial communities and invertebrates likely ripples through the food web as it
304 forms a key source of fine particulate OM for filter- and deposit feeding invertebrates
305 (Bundschuh & McKie, 2016). Impaired invertebrate growth rates may also lead to reduced
306 biomass available for predators that rely on invertebrate shredders as food source, such as fish
307 (Rask & Hiisivuori, 1985) and invertebrate predators (Herrmann, 1984; Krisp & Maier, 2005).
308 In other words, current monitoring efforts that rely on single time point estimation of
309 invertebrate abundances fail to capture the temporal nature and complexity of trophic
310 interactions (e.g., knock-on effects on growth rate and other fitness variables) that ultimately
311 govern the functioning and health of ecosystems. Thus, the likelihood that monitoring efforts
312 can lead to misinterpretations of ecosystem health assessments calls for reconsidering our
313 approaches in biodiversity assessments and environmental diagnostics.

314 **Conclusion**

315 Using OM that encapsulates the naturally relevant complexity of agricultural chemicals
316 entering adjacent water bodies, this study shows that agricultural practices can reduce the
317 contribution of microbial communities to OM degradation, which can coincide with a reduction
318 in growth rates of the aquatic invertebrate *A. aquaticus* as assessed under controlled laboratory
319 conditions. Quantifying length of *A. aquaticus* in the field in ditches adjacent to different
320 agricultural practices reveals that the patterns observed in our laboratory study reflect those

321 occurring in natural systems. In a context of natural complexity and interdependence of
322 ecological processes, these results suggest that agricultural chemicals can impair trophic
323 linkages between OM-associated microbial communities and invertebrates.

324

325 **Ethics approval and consent to participate**

326 Not applicable.

327

328 **Consent for publication**

329 Not applicable.

330

331 **Availability of data and material**

332 The datasets and/or scripts used for data analysis are available upon request.

333

334 **Competing interests**

335 The authors state that they have no conflict of interest.

336

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339

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