Microplastic leachates induce species-specific trait strengthening in intertidal mussels

Laurent Seuront[a,b,c*], Katy R. Nicastro[c,d], Christopher D. McQuaid[c], Gerardo I. Zardi[c]

[a] *Univ. Lille, CNRS, Univ. Littoral Côte d'Opale, UMR 8187 – LOG – Laboratoire d'Océanologie et de Géosciences, F-59000 Lille, France*

[b] *Department of Marine Resource and Energy, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan*

[c] *Department of Zoology and Entomology, Rhodes University, Grahamstown, 6140, South Africa*

[d] *CCMAR–Centro de Ciencias do Mar, CIMAR Laboratório Associado, Universidade do Algarve, Campus de Gambelas, Faro, 8005-139, Portugal*

* Corresponding Author: Laurent Seuront, Email: laurent.seuront@cnrs.fr

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Abstract

Plastic pollution is ubiquitous with increasing recognition of its direct effects on species' fitness. Little is known, however, about its more subtle effects, including the influence of plastic pollution on the morphological, functional and behavioural traits of organisms that are central to their ability to withstand disturbances. Among the least obvious but most pernicious forms of plastic-associated pollution are the chemicals that leach from microplastics. Here, we investigate how such leachates influence species' traits by assessing functional trait compensation across four species of intertidal mussels, through investigations of byssal thread production, movement and aggregation behaviour for mussels held in natural seawater or seawater contaminated by microplastic leachates.We found no evidence for compensation of functional traits, but for each species, microplastic leachates reinforced one trait while others remained unaffected. Two species (*Perna perna* and *Mytilus galloprovincialis*), were characterized by a resistance strategy to disturbance; they produced more byssal threads in microplastic leachate seawater than in control seawater, while motility and aggregation remained essentially unaffected. In contrast, the other two species (*M. edulis* and *Choromytilus meridionalis*), showed a resilience strategy to disturbance through reduced motility and aggregation in leachate seawater, while byssal thread production remained unaffected. These results suggest that the competitive abilities of intertidal mussels may be related to their sensitivity to microplastic leachates or other chemical disturbance. Importantly, the trait strengthening observed will affect the ability of these mussels to form spatially patterned beds, with implications for their quality as autogenic ecological engineers or foundation species. Thus, our findings have implications for the ability of mussel beds to tolerate disturbance, and hence for central ecosystem services, such as their ability to support biodiversity and enhance secondary and tertiary production. The results suggest that an inconspicuous aspect of plastic pollution has the potential to influence other communities and ecosystems in powerful ways.

Keywords

Trait compensation, Trait strengthening, Aggregation, Movement, Microplastic pollution, Microplastic leachates, Chemical contamination, Resilience, Disturbance, Resistance

INTRODUCTION

Trait-based approaches provide a valuable framework to understand how species, populations and communities respond to environmental variation, and the consequences for ecosystem functioning (Lavorel and Garnier 2002, Moretti et al. 2009, Cornwell and Ackerly 2009, Lavorel et al. 2011, Dray et al. 2014, Jung et al. 2014, Simons et al. 2016). Within this context, animal behaviour is usually seen as more flexible than the morphological traits typically used in *e.g.* trait-based plant community ecology (Pigliucci 2001, Duckworth 2008, Sih et al. 2010) and trait-based predator-prey interactions in both invertebrates (Cotton et al. 2004, Teplitsky and Laurila 2007, Dahirel et al. 2017) and vertebrates (Relyea 2001, 2003, Kuo et al. 2015). The ability of organisms to respond to both natural environmental changes (Wellstein et al. 2011, Jung et al. 2014) and various forms of anthropogenically-induced rapid environmental changes such as urbanisation (Sih et al. 2010, Dahirel et al. 2017) are mediated by their functional traits. Environmental changes caused by increasing levels of plastic pollution should be no exception and ecosystem responses will similarly depend on trait-based responses.

Plastics are increasingly recognised as one of the most ubiquitous and conspicuous sources of pollution of the Anthropocene, especially in the marine environment (Li et al. 2016), and constitute a global challenge to ocean governance (Vince and Hardesty 2017, Haward 2018). Beyond the conspicuous impacts of plastic pollution such as the deposition of beached debris (Barnes et al. 2009, Browne et al. 2011) and the entanglement of marine fauna (Gall and Thompson 2015), microplastics (i.e. plastic particles < 5 mm) are now considered the most numerically abundant form of solid waste on Earth (Eriksen et al., 2014) and a potential threat to marine ecosystems globally (Galloway et al. 2017). The adverse effects of microplastics on marine life form a very active area of research, see *e.g.* Galloway et al. (2017), de Sá et al. (2018) and Anbumani and Kakkar (2018) for recent reviews, and to date, one of the most scrutinised marine organisms in a microplastic context is the blue mussel *Mytilus edulis*, which has even been referred to as a "*sentinel for monitoring microplastic pollution*" (Bråte et al. 2018). Like other benthic filter feeders, such as the Pacific oyster *Crasostera gigas*, the European flat oyster *Ostrea edulis* and the Manila clam *Venerupis philippinarum*, *M. edulis* ingests and accumulates microplastic particles and fibers (von Moos et al. 2012, Van Cauwenberghe et al. 2015, Catarino et al. 2018, Li et al. 2016, 2018) that act as vectors of the chemical pollutants adsorbed onto their surface. Their ingestion prompts the desorption of these chemicals with immediate adverse effects (Avio et al. 2015, Lo and Chan 2018, Leung and Chan 2018), but they also accumulate in the tissues and organs, and ultimately cascade upwards through the food chain (Desforges et al. 2015). Microplastics are particularly problematic in aquatic environments because they release additives used in their manufacture (Fries and Zarfl 2011), while contaminants accumulate more readily and persist longer on their surfaces than on large plastic debris (Law and Thompson 2014, Bejgarn et al. 2015).

Our understanding of the effects of microplastic leachates on marine invertebrates is still in its infancy but they have been shown to impair a range of functional traits including embryonic development in the sea urchin *Lytechinus variegatus* (Nobre et al. 2016) and the clam *Meretrix meretrix* (Ke et al. 2019), posterior segment regeneration in the polychaete *Perinereis aibuhitensis* (Leung and Chan 2017), and both growth and development in the false limpet *Crepidula onyx* (Lo and Chan 2018). Microplastic leachates also lead to the impairment and eventual inhibition of behavioural traits such as vigilance and predator avoidance in the intertidal gastropod *Littorina littorea* (Seuront 2018). Other studies have shown effects of both biodegradable and conventional microplastics on the oyster *Ostrea edulis* (Green 2016), the impairment of embryonic development in the brown mussel *Perna perna* (Gandara e Silva et al. 2016) and the Mediterranean mussel *Mytilus galloprovincialis* (Capolupo et al. 2020), and a reduction of ~50% in byssal thread production and attachment strength in mussels (*Mytilus edulis*) exposed to polyethylene microplastics (Green et al., 2019).

The last two examples are important because intertidal mussels are key ecosystem engineers that influence coastal species richness by modifying habitats, increasing spatial complexity and facilitating the presence of other species (Cole and McQuaid 2010). They typically exhibit two sets of adaptive behavioural functional traits, *i.e.* (i) the number and strength of byssal threads (extensible proteinaceous fibres used for attachment to the substratum), and (ii) motility and the ability to aggregate. These traits both contribute to the creation and maintenance of dynamic biogenic beds (Schneider et al. 2005, Liu et al. 2014) that are resistant to both predation (Day et al. 1991, Côté 1995, Leonard et al. 1999) and hydrodynamic stress (Zardi et al. 2006). More importantly, mussels respond to stress, e.g. predators and predation cues, through an increase in traits such as the number and strength of byssal threads, motility, and aggregation (Nicastro et al. 2007, Kobak and Kakareko 2009, Commito et al. 2016) though exceptions do exist (Ishida & Iwasaki 2003). The phenomenon of trait compensation, is often exemplified by the relationship between morphological and behavioral defense traits. For example, morphologically more vulnerable individuals tend to display a higher degree of predator avoidance than those that are less vulnerable (e.g. Rundle and Bronmark 2001; Cotton et al. 2004, Mikolajewski and Johansson 2004). More generally, however, trait complensation describes how individuals can offset the costs of one trait with the benefits of another one (DeWitt et al. 1999). Examples of trait compensation are still scarce in intertidal marine bivalves (Seed and Brown 1978, Commito et al. 1982, Bertness and Grosholz 1985), though a typical example of functional trait compensation is found in intertidal mussels where species characterized by strong byssal attachment typically move and aggregate less than species that attach weakly but exhibit greater mobility. Physical disruption of mussel beds, especially through wave action, is a major cause of mortality such trait compensation allows species to adopt either a resistance or a resilience approach to minimising disruption of the collective bed (Nicastro et al. 2007, 2008). Such compensatory effects are increasingly recognized as underlying the evolutionary trajectories of suites of fitness-related traits (Husak and Swallow 2011, Dennenmoser and Christy 2013, Lailvaux et al. 2014).

In this context, we examine the effects of exposure to leachates from raw polypropylene pellets on the relationships among byssal thread production and both movement and aggregation behavior in four species of intertidal mussels. Specifically, we assessed if byssal thread production and movement and aggregation are negatively correlated across species, i.e. whether there would be interspecific differences in trait compensation, and whether leachates would affect each species' suite of traits differently.

MATERIALS AND METHODS

Study organisms

We used four species of intertidal mussels: the blue mussels *Mytilus edulis* (Linnaeus, 1758) and *M. galloprovinciallis* (Lamarck, 1819), the southern African black mussel, *Choromytilus meridionalis* (Krauss, 1848), and brown mussel, *Perna perna* (Linnaeus, 1758).

M. edulis was collected in October 2018 from the Pointe aux Oies, a rocky intertidal reef hosting a large mussel bed located along the French coast of the eastern English Channel (50°47'12N, 1°36'12E). *M. galloprovinciallis* and *P. perna* were sampled in November 2018 from Shark Rock, an intertidal South African rocky shore where the two species coexist (33°58'53"S 25°39'49"E). *C. meridionalis* was collected intertidally from Port Alfred (33°36'13.0"S 26°54'06.3"E) in South Africa in December 2018.

Because the ambient levels of plastic leachates may differ between French and South African waters, we used the density of microplastic particles smaller than 5 mm found in the high-tide mark surface sediments of beaches surrounding our sampling sites as a proxy of microplastic seawater contamination. Microplastic items, including beached pellets, were collected from ten quadrats (20 cm × 20 cm) haphazardly placed along a 50-meter stretch of the high-tide mark and subsequently enumerated. Microplastic density did not significantly differ between France and South Africa (Wilcoxon-Mann-Whitney test, $p > 0.05$), and consistently ranged from 710 to 955 microplastic particles per meter square, including 250 to 450 beached pellets.

Prior to the experiments, mussels were acclimatized for 24 hours under a natural light cycle in glass aquaria filled with aerated seawater under standardized conditions of salinity (S = 35 PSU) and temperature (T = 18°C) similar to the field conditions, i.e. S = 33 PSU and T = 16°C for *M. edulis* and S = 35 PSU and T = 18°C for *M. galloprovincialis*, *P. perna* and *C. meridionalis*.

Microplastic leachate treatment

Individuals of each species were exposed to either control seawater or microplastic leachate seawater. Control seawater consisted of natural seawater collected from the sampling sites of each species. Microplastic leachate seawater was prepared from commercially available virgin polypropylene pellets (typically 3.3 to 4.7 mm in diameter; Pemmiproducts, Aachen, Germany) mixed with control seawater at a concentration of 20 ml of pellets (ca. 600 pellets) per liter and aerated for 24 h before the beginning of the behavioral assays (Seuront 2018). Virgin pellets were specifically chosen instead of beached pellets as the latter accumulate various persistent organic pollutants, polycyclic aromatic hydrocarbons and heavy metals onto their surfaces at concentrations higher than those found in the environment (Fries and Zarfl 2011). As a result, the leachate from beached pellets is likely to contain a more complex mixture of contaminants, at higher and highly variables concentrations, than the leachate from virgin pellets. Although not quantified in this experiment, polypropylene leachates typically contain bisphenol A, phthalates, octylphenol, nonylphenol and brominated flame retardants (Sánchez-Avila et al. 2012, Hermabessiere et al. 2017, Vered et al. 2019) and have recently been shown to be far less toxic to various *M. galloprovincialis* traits than leachates produced from car tire rubber, polyethylene terephthalate, polystyrene and polyvinyl chloride microplastics (Capolupo et al. 2020).

Aggregation behavior and crawling distance

All behavioral experiments were run in 26 cm diameter glass arenas with smooth, featureless surfaces under static conditions to avoid passive movement of mussels by water currents (Nicastro et al. 2007, Commito et al. 2014, 2016). In each arena, 2L of either microplastic leachate or control water was used. Fifteen mussels, 3-4 cm shell length, were placed on the bottom of each arena, with mussel centers 3.5 cm equidistant from each other in concentric circles and the anterior narrow end facing the center of the arena. To assess the presence of (i) a leachate effect on each species, and (ii) differences in the response to microplastic leachate between species, we ran a series of single-species trials, pairing leachate versus control. More specifically, for each species, 3 control and 3 leachate treatments were run simultaneously, and replicated on two different days ($n = 6$).

Mussel positions were recorded every 5 minutes for 8 hours using a GoPro camera (GoPro HERO7 Black) placed 40 cm above the arenas. Mussels were subsequently classified as either solitary (i.e. isolated individuals that did not become part of an aggregation) or aggregated (i.e. in a group of 2 or more mussels with their shells in physical contact with each other; Nicastro et al. 2007, Commito et al. 2016). Aggregation behavior was quantified at each 5 min interval as the proportion of mussels (%), forming aggregates. The number of aggregates and the number of individuals in each aggregate were recorded after 8 hours. The proportion of aggregated mussels was expressed as a function of time t, and fitted with a nonlinear equation of the form $A(t) = A_{\max}t/(k + t)$, where, by analogy with the Michaelis-Menten equation, $A_{\max}$ (%) and k (min) are empirical parameters describing the maximal proportion of aggregation and the time when the proportion of aggregation was half of the maximum value (i.e. $A(t) = A_{\max}/2$), respectively. For each species, the parameters $A_{\max}$ and k were estimated for control and treatment experiments using a nonlinear least-squares Levenberg–Marquardt algorithm, and were chosen as the values that respectively maximized the coefficient of determination r^2 and minimized the sum of the squared residuals among empirical data (Seuront 2013). The total distance crawled d_c was recorded for each individual over the first 3 h of each experiment (Nicastro et al. 2007).

Byssal thread production

For each treatment (control or leachate seawater), mussels (n =17 for *M. edulis* and n = 11 for the other species) were divided among four separate 2 L glass tanks. Individuals were placed on concrete tiles, at least 10 cm apart so that they maintained a solitary position (i.e. horizontal to the substratum) and attachment strengths were not influenced by the physical contact of nearby conspecifics but only by background water-born chemical cues. Byssal threads attach to the substratum by means of a proteinaceous plaque and after 8h, byssal thread secretion was quantified. Mussel attachment is a dynamic rather than static process involving a continuous turnover of byssal threads (Lee et al. 1990) with only a portion of threads remaining in use after a period of a few hours (Allen et al. 1976). As such, and because different species have different thread attach-detach rates, we quantified byssal thread secretion as the total number of plaques, N, (connected or unconnected to the byssal stem) adhered to the tile.

Statistical analyses

To take the potential confounding effect of replicating our single-species trials on different days into account explicitly, we first assessed the presence of differences in $A_{\max}$, k, aggregate size and number, crawling distances, and the number of byssal plaques observed in control seawater and microplastic leachate seawater using the Wilcoxon-Mann-Whitney test for each species. As no significant differences ($p > 0.05$) were found between replicates for any of the four mussel species investigated, the behavioral data were pooled for further analysis. The presence of differences in $A_{\max}$, k, aggregate size and number, crawling distances, and the number of byssal plaques between control seawater and microplastic leachate seawater was subsequently assessed for each species using the Wilcoxon paired-sample test (Zar 2009). Multiple comparisons between the behavioral responses of different species to microplastic leachate seawater were conducted using the Kruskal-Wallis test, and a subsequent multiple comparison procedure based on the Tukey test was used to identify distinct groups of measurements (Zar 2009).

RESULTS

Aggregation behavior

The percentages of aggregated individuals $A(t)$ followed a similar temporal pattern for all species (Fig. 1). They were consistently characterized by a sharp increase over the first 3 hours of the experiments before reaching a plateau where they were nearly constant. Clear differences in percentages of aggregated mussels were observed between control and leachate seawater in *M. edulis* and *C. meridionalis* (Fig. 1): $A(t)$ was consistently significantly different ($p < 0.01$) after $t = 5$ minutes in *M. edulis* and $t = 15$ minutes for *C. meridionalis*. In both species, $A(t)$ was significantly higher in microplastic leachate seawater ($p < 0.01$). In contrast, with a few exceptions, *M. galloprovincialis* and *P. perna* did not exhibit any significant differences ($p > 0.05$) between their $A(t)$ in control and leachate seawater (Fig. 1).

The temporal evolution of the percentage of aggregated individuals rate $A(t)$ was consistently described by the relation $A(t) = A_{\max}t/(k + t)$, with the coefficient of determination r^2 ranging from 0.88 to 0.97 in control seawater and from 0.91 to 0.96 in microplastic leachate seawater. The resulting values of the fitting parameters $A_{\max}$ (Fig. 2a) and k (Fig. 2b) exhibited significant differences among species in both control seawater and leachate seawater. Thus, microplastic leachates significantly ($p < 0.05$) increased the proportion of mussels aggregating (A_{max}, Fig. 2a) and decreased the time taken to aggregate (k, Fig. 2b for *M. edulis* and *C. meridionalis* with no effect on *P. perna* or *M. galloprovincialis* except for a decrease in k for *M. galloprovincialis*.

Finally and for all species, treatment had no significant effect ($p > 0.05$) on aggregate number or aggregate size which ranged between 0 and 4 aggregates of 2 to 7 individuals in control seawater and between 1 and 4 aggregates of 2 to 7 individuals in leachate seawater.

Crawling distance

Significant differences in crawling distances were consistently observed between species ($p < 0.05$, Fig. 3), with d_c(*P. perna*) < d_c(*M. galloprovincialis*) < d_c(*M. edulis*) < d_c(*C. meridionalis*) in both control seawater and leachate seawater. Significant differences between control and leachate seawater were observed in *M. edulis* and *C. meridionalis*, which moved significantly more in leachate seawater than in control seawater. In contrast, *P. perna* and *M. galloprovinciallis* did not show any significant difference in crawling distance between control and leachate seawater.

Byssal thread production

Significant differences in the number of byssal plaques (N) were consistently observed between species ($p < 0.05$, Fig. 4), with N(*P. perna*) = N(*M. galloprovincialis*) = N(*M. edulis*) < N(*C. meridionalis*) in control seawater, and N(*M. edulis*) < N(*P. perna*) < N(*M. galloprovincialis*) = N(*C. meridionalis*) in leachate seawater. Microplastic leachate seawater did not significantly affect the number of byssal threads produced by *M. edulis* or *C. meridionalis* ($p > 0.05$, Fig. 4). In contrast, *P. perna* and *M. galloprovincialis* individuals produced significantly more byssal threads when exposed to leached water than under control conditions ($p < 0.001$).

DISCUSSION

Our results clearly indicate that microplastic leachates consistently led to trait strengthening (Table 1). Importantly, strengthening of one trait under leachate stress was not accompanied by any evidence of trait compensation among byssal thread production, movement and aggregation. Thus, microplastic leachates reinforced one trait in each species, while the others remained unaffected. Noticeably, the responses differed between two groups of mussels that are described hereafter as exhibiting either resilience or resistance responses to disturbance. Here, resilience specifically refers to the capacity of a mussel bed to respond to environmental perturbations though reorganization of the spatial properties of the bed (de Paoli et al. 2017), while resistance indicates strengthening of the bed structure that remains essentially unchanged (Nicastro et al. 2007, 2008). Two species (i.e. *P. perna* and *M. galloprovincialis*) showed a resistance strategy to disturbance through the production of more byssal threads in microplastic leachate seawater than in control seawater, while motility and aggregation essentially remained unaffected. In contrast, the other two species (*M. edulis* and *C. meridionalis*) exhibited a resilience strategy to disturbance, i.e. they showed increased motility and aggregation, while thread production remained unaffected. Interestingly, one species from each pair is native from Europe and one from South Africa, which further suggests that resilience and resistance are shared strategies within each region.

These results support previous evidence of the negative effects of microplastics and microplastic leachates on intertidal organisms, including mussels (Gandara e Silva et al. 2016, Seuront 2018, Capolupo et al. 2020). They contrast, however, with recent evidence of reduced byssal thread production and attachment strength in *M. edulis* following prolonged exposure to polyethylene microplastics (Green et al. 2019). This apparent discrepancy may, however, relate to both the differences in exposure time and plastic considered, especially as polyethylene leachate have recently been shown (Capolupo et al. 2000) to be more toxic than the polypropylene leachate used in the present work. Our results also build upon previous work by showing that the stress induced by microplastic leachates reinforces specific traits in different species. The absence of trait compensation is particularly critical given that the importance of phenotypic variation has generally been underscored by calls for a mechanistic (i.e. physiological) understanding of climate change impacts (e.g. Helmuth et al. 2005, 2006, Chown et al. 2010). Understanding how populations react to rare/novel environments is, however, an absolute prerequisite if we are to predict their fates accurately over short and long timescales (Sheldon and Dillon 2016). In this context, our results are critical to assessing the ability of intertidal mussel populations to withstand natural and anthropogenic disturbances, both of which are expected to increase in the coming decades (IPCC 2018). Intertidal mussels are important ecosystem engineers through their attachment to the substratum in dense mono- or multi-layered beds that create microhabitats that remain moist and thermally benign during low tide and offer protection against wave action during high tide (Little et al. 2008). By influencing either byssal attachment strength or the ability to move and aggregate, the trait reinforcement observed in the present work is likely to affect the formation and maintenance of spatial patterns in mussel beds, which are a key feature in the ability of mussels to withstand disturbances (Rietkerk and van de Koppel 2008, Pringle et al. 2010).

Offsetting the costs of one trait with the benefits of another through trait compensation (Seed and Brown 1978, Commito et al. 1982, Bertness and Grosholz 1985) is believed to shape the evolution of suites of potentially antagonistic traits that influence fitness. Such offsetting highlights the complexity of the relationship between performance and other traits (Lailvaux and Husak 2014), but in this case we found no evidence for short-term compensation. Because mussel beds modify habitat complexity and facilitate the establishment and persistence of a wide variety of associated invertebrates (Palomo et al. 2007, Arribas et al. 2013, 2014), pattern formation has ecosystem consequences well beyond species fitness. Our results indicate that behaviors that affect the ability to form patterns are influenced by microplastic leachates in species-specific ways that differed between two groups of species. The effects of leachates and other chemicals on the traits investigated will also affect competitive interactions between mussels and other space-occupying organisms (including competing mussel species). Species showing enhanced motility and faster aggregation when exposed to this form of pollution (i.e. *M. edulis* and *C. meridionalis*) are likely to exhibit more rapid pattern formation in the face of disturbance and to be at a competitive advantage, with positive effects on central ecosystem services, such as biodiversity and both secondary and tertiary production. It is possible that intrinsic and extrinsic drivers such as age and food abundance will affect the expression of compensatory traits (Kuo et al. 2015), but our results suggest that such expression can have effects that will extend beyond the individual species.

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Literature Cited

Allen, J. A., Cook M., Jackson D. J., Preston S., and Worth E. M. 1976. Observations on the rate of production and mechanical properties of the byssus threads of *Mytilus edulis* L. Journal Molluscan Studies 42,279-289.

Anbumani, S., and P. Kabbar. 2018. Ecotoxicological effects of microplastics on biota: a review. Environmental Science and Pollution Research 25:14373-14396.

Arribas, L.P., M. Bagur, E. Klein, P. Penchaszadeh, and M. G. Palomo. 2013. Geographic distribution of mussel species and associated assemblages along the northern Argentinean coast. Aquatic Biology 18:91-103.

Arribas, L. P., L. Donnarumma, M. G. Palomo, and R. A. Scrosati. 2014. Intertidal mussels as ecosystem engineers: their associated invertebrate biodiversity under contrasting wave exposure. Marine Biodiversity 44, 203-211.

Avio, C. G., S. Gorbi, M. Milan, M. Benedetti, D. Fattorini, G. d'Errico, M. Pauletto, L. Bargelloni, and F. Regoli. 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environmental Pollution 198:211-222.

Barnes, D.K.A., F. Galgani, R. C. Thompson, and M. Barlaz. 2009. Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society, B 364:1985–1998.

Bejgarn, S., M. MacLeod, C. Bogdal, and M. Breitholtz. 2015. Toxicity of leachate from weathering plastics: an exploratory screening study with *Nitocra spinipes*. Chemosphere 132:114-119.

Bertness, M.D., and E. Grosholz. 1985. Population dynamics of the ribbed mussel, *Geukensia demissa*: The costs and benefits of an aggregated distribution. Oecologia 67:192-204.

Bråte, I. L. N., R. Hurley, K. Iversen, J. Beyer, K. V. Thomas, C. C. Steindal, N. W. Green, M. Olsen, and A. Lusher. 2018. *Mytilus* spp. as sentinels for monitoring microplastic pollution in Norwegian coastal waters: A qualitative and quantitative study. Environmental Pollution 243:383-393.

Browne, M.A., P. Crump, S. J. Nivens, E. Teuten, A. Tonkin, T. Galloway, and R. Thompson. 2011. Accumulation of microplastics on shorelines worldwide: sources and sinks. Environmental Science and Technology 45:9175–9179.

Capolupo, M., L. Sørensen, K.D.R. Jayasena, A.M. Booth, and E. Fabbri. 2020. Chemical composition and ecotoxicity of plastic and car tire rubber leachates to aquatic organisms. Water Research 169:115270.

Catarino, A. I., V. Macchia, W. G. Sanderson, R. C. Thompson, and T. B. Henry. 2018. Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. Environmental Pollution 237:675-684.

Chown, S. L., A. A. Hoffmann, T. N. Kristensen, M. J. Angilletta Jr., N. Chr. Stenseth, and C. Pertoldi. 2010. Adapting to climate change: a perspective from evolutionary physiology. Climate Research 43:3-15.

Cole, V. J., and C. D. McQuaid. 2010. Bioengineers and their associated fauna respond differently to the effects of biogeography and upwelling. Ecology 91:3549-3562.

Commito, J. A. 1982. Effects of *Lunatia heros* predation on the population dynamics of *Mya arenaria* and *Macoma balthica* in Maine, USA. Marine Biology 69:187-193.

Commito, J. A., A. E. Commito, R. V. Platt, B. M. Grupe, W. E. Dow Piniak, N. J. Gownaris, K. A. Reeves, and A. M. Vissichelli. 2014. Recruitment facilitation and spatial pattern formation in soft-bottom mussel beds. Ecosphere 5:160.

Commito, J. A., N. J. Gownaris, D. E. Haulsee, S. E. Coleman, and B. F. Beal. 2016. Separation anxiety: mussels self-organize into similar power-law clusters regardless of predation threat cues. Marine Ecology Progress Series 547:107-119.

Cornwell, W. K., and D. D. Ackerly. 2009. Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. Ecological Monographs 79:109–126.

Côté, I. M. 1995. Effects of predatory crab effluent on byssus pro- duction in mussels. Journal of Experimental Marine Biology and Ecology 188:233-241.

Cotton, P. A., S. D. Rundle, and K. E. Smith. 2004. Trait compensation in marine gastropods: shell shape, avoidance behavior, and susceptibility to predation. Ecology 85:1581-1584.

Dahirel, M., J. Dierick, M. De Cock, and D. Bonte. 2017. Intraspecific variation shapes community-level behavioral responses to urbanization in spiders. Ecology 98:2379-2390.

Day, R.W., A. Barkai and P.A. Wickens. 1991. Trapping of three drilling whelks by two species of mussels. Journal of Experimental Marine Biology and Ecology 149: 109-122.

De Sá, L. C., M. Oliveira, F. Ribeiro, T. L. Rocha, and M. N. Futter. 2018. Studies of the effects of microplastics on aquatic organisms: What do weknow and where should we focus our efforts in the future? Science of the Total Environment 645:1029-1039.

Dennenmoser, S., and J. H. Christy. 2013. The design of a beautiful weapon: compensation for opposing sexual selection on a trait with two functions. Evolution 67:1181-1188.

Desforges, J., M. Galbraith, and P. S. Ross. 2015. Ingestion of microplastics by zooplankton in the northeast Pacific Ocean. Archives of Environmental Contamination and Toxicology 69:320-330.

DeWitt, T. J., A. Sih, and J. A. Hucko. 1999. Trait compensation and cospecialization in a freshwater snail: size, shape and antipredator behaviour. Animal Behaviour 58:397– 407.

Dray, S., P. Choler, S. Dolédec, P. R. Peres-Neto, W. Thuiller, S. Pavoine, and C. J. F. ter Braak. 2014. Combining the fourth-corner and the RLQ methods for assessing trait responses to environmental variation. Ecology 95:14–21.

Duckworth, R. A. 2008. The role of behavior in evolution: a search for mechanism. Evolutionary Ecology 23:513–531.

Eriksen, M., L. C. M. Lebreton, H. S. Carson, M. Thiel, C. J. Moore, J. C. Borerro, F. Galgani, P. G. Ryan, and J. Reisser. 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One 9:e111913.

Fries, E., and C. Zarfl. 2011. Sorption of polycyclic aromatic hydrocarbons (PAHs) to low and high density polyethylene (PE). Environmental Science and Pollution Research 19:1296-1304.

Gall, S.C., and R. C. Thompson. 2015. The impact of debris on marine life. Marine Pollution Bulletin 92:170–179.

Galloway, T. S., M. Cole, and C. Lewis. 2017. Interactions of microplastic debris throught the marine ecosystem. Nature Ecology and Evolution 1:0116.

Gandara e Silva, P.P., C. R. Nobre, P. Resaffe, C. D. S. Pereira, and F. Gusmao. 2016. Leachate from microplastics impairs larval development in brown mussels. Water Research 106:364-370.

Green, D. S. 2016. Effects of microplastics on European flat oysters, *Ostrea edulis* and their associated benthic communities. Environmental Pollution 216:95-103.

Green, D. S., T. J. Colgan, R. C. Thompson, and J. C. Carolan. 2019. Exposure to microplastics reduces attachment strength and alters the haemolymph proteome of blue mussels (*Mytilus edulis*). Environmental Pollution 246:423-434.

Haward, M. 2018. Plastic pollution of the world's seas and oceans as a contemporary challenge in ocean governance. Nature Communications 9:667.

Helmuth, B., J. G. Kingsolver, and E. Carrington, E. 2005. Biophysics, physiological ecology, and climate change: Does mechanism matter? Annual Review of Physiology 67:177-201.

Helmuth, B., N. Mieszkowska, P. Moore, and S. J. Hawkins. 2006. Living on the edge of two changing worlds: forecasting the responses of rocky intertidal ecosystems to climate change. Annual Review of Ecology, Evolution, and Systematics 37:373-404.

Hermabessiere, L., A. Dehaut, I. Paul-Pont, C. Lacroix, R. Jezequel, P. Soudant, and G. Duflos. 2017. Occurrence and effects of plastic additives on marine environments and organisms: a review. Chemosphere 182:781–793

Husak, J., and J. Swallow. 2011. Compensatory traits and the evolution of male ornaments. Behaviour, 148:1-29.

Ishida, S., and K. Iwasaki. 2003. Reduced byssal thread production and movement by the intertidal mussel *Hormomya mutabilis* in response to effluent from predators. Journal of Ethology 21:117-122.

Jung, V., C. H. Albert, C. Violle, G. Kunstler, G. Loucougaray, and T. Spiegelberger. 2014. Intraspecific trait variability mediates the response of subalpine grassland communities to extreme drought events. Journal of Ecology 102:45–53.

Ke, A. Y., et al. 2019. Impacts of leachates from single-use polyethylene plastic bags on the early development of clam *Meretrix meretrix* (Bivalvia: Veneridae). Marine Pollution Bulletin 142:54-57.

Kobak, J., and T. Kakareko. 2009. Atachment strength, aggregation and movement of the zebra mussel (Dreissena polymorpha, Bivalvia) in the presence of potential predators. Fundamental and Applied Limnology 174:193-204.

Kuo, C. Y., D. J. Irschick, and S. P. Lailvaux. 2015. Trait compensation between boldness and the propensity for tail autotomy under different food availabilities in similarly aged brown anole lizards. Functional Ecology 26:385-392.

Lailvaux, S. P., and J. F. Husak. 2014. The life history of whole- organism performance. Quarternary Reviews in Biology 89: 285-318.

Lailvaux, S.P., R. Wilson, and M. M. Kasumovic. 2014. Trait compensation and sex-specific aging of performance in male and female professional basketball players. Evolution 68:1523-1532.

Lavorel, S., and E. Garnier. 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Functional Ecology16:545–556.

Lavorel, S., K. Grigulis, P. Lamarque, M.-P. Colace, D. Garden, J. Girel, G. Pellet, and R. Douzet. 2011. Using plant functional traits to understand the landscape distribution of multiple ecosystem services. Journal of Ecology 99:135–147.

Law, K. L., and R. C. Thompson. 2014. Microplastics in the seas. Science 345:144-145.

Lee C. Y., Lim S. S. L., Owen M.O. 1990. The rate and strength of byssal reattachment by blue mussels (*Mytilus edulis* L.). Canadian Journal of Zoology 68:2005-2009.

Leonard, G. H., M. D. Berteness, and P. O. Yund. 1999. Crab predation, waterborne cues, and inducible defences in the blue mussel, *Mytilus edulis*. Ecology 80:1-14.

Leung, J., and K. Y. K. Chan. 2018. Microplastics reduced posterior segment regeneration rate of the polychaete *Perinereis aibuhitensis*. Marine Pollution Bulletin 129:782-786.

Li, W. C., H. F. Tse, and L. Fok. 2016. Plastic waste in the marine environment: A review of sources, occurrence and effects, Science of the Total Environment, 566–567:333-349.

Li, J. C. Green, A. Reynolds, H. Shi, and J. M. Rotchell. 2018. Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom. Environmental Pollution 241:35-44.

Li, J., X. Qu, W. Zhang, D. Yang, P. Kolandhasamy, D. Li, and H. Shi. 2016. Microplastics in mussels along the coastal waters of China. Environmental Pollution 214:177-184.

Little, C., G. A. Williams, and C. D. Trowbridge. 2008. The biology of rocky shores. Oxford University Press, Oxford (2008).

Liu, Q. X., P. M. J. Herman, W. M. Mooij, J. Huisman, M. Scheffer, H. Off, and J. van de Koppel. 2014. Pattern formation at multiple spatial scales drives the resilience of mussel bed ecosystems. Nature Communications 5:5234.

Lo, H. K. A., and K. Y. K. Chan. 2018. Negative effects of microplastic exposure on growth and development of *Crepidula onyx*. Environmental Pollution 233:588-595.

Mikolajewski, D. J., and F. Johansson. 2004. Morphological and behavioral defenses in dragonfly larvae: trait compensation and cospecialization. Behavioral Ecology 15:614-620.

Moretti, M., F. De Bello, S. P. M. Roberts, and S. G. Potts.2009. Taxonomical vs. functional responses of bee communities to fire in two contrasting climatic regions. Journal of Animal Ecology 78:98–108.

Nicastro, K. R., G. I. Zardi, and C. D. McQuaid. 2007. Behavioural response of invasive *Mytilus galloprovincialis* and indigenous *Perna perna* mussels exposed to risk of predation. Marine Ecology Progress Series 336:169-175.

Nicastro, K. R., G. I. Zardi, and C. D. McQuaid. 2008. Movement behaviour and mortality in invasive and indigenous mussels: resilience and resistance strategies at different spatial scales. Marine Ecology Progress Series 372:119-126.

Nobre, C.R., M. F. M. Santana, A. Maluf, F. S. Cortez, A. Cesar, C. D. S. Pereira, and A. Turra. 2015. Assessment of microplastic toxicity to embryonic development of the sea urchin *Lytechinus variegatus* (Echinodermata: Echinoidea). Marine Pollution Bulletin 92:99-104.

Palomo, M.G., J. People, M. G. Chapman, and A. J. Underwood. 2007. Separating the effects of physical and biological aspects of mussel beds on their associated assemblages. Marine Ecology Progress Series 344:131-142.

Petraitis, P. S. 1987. Immobilization of the predatory gastropod, *Nucella lapillus,* by its prey, *Mytilus edulis*. Biological Bulletin 172:307-314.

Pigliucci, M. 2001. Phenotypic plasticity: beyond nature and nurture. Johns Hopkins University Press, Baltimore, Maryland, USA.

Pringle, R. M., D. F. Doak, A. K. Brody, R. Jocqué, and T. M. Palmer. 2010. Spatial pattern enhances ecosystem functioning in an African savanna. PLoS Biology 8:e1000377.

Relyea, R. A. 2001. The relationship between predation risk and antipredator responses in larval anurans. Ecology 82:541-554.

Relyea, R. A. 2003. Predators come and predator go: the reversibility of predator-induced traits. Ecology 84:1840-1848.

Rietkerk, M., and J. van de Koppel. 2008. Regular pattern formation in real ecosystems. Trends in Ecology and Evolution 23:169-175.

Rundle, S. D., and C. Brönmark. 2001. Inter- and intraspecific trait compensation of defence mechanisms in freshwater snails. Proceedings of the Royal Society of London, Series B 268:1463–1468.

Sánchez-Avila, J., R. Tauler, and S. Lacorte. 2012. Organic micropollutants in coastal waters from NW Mediterranean Sea: sources distribution and potential risk. Environment International 46:50-62.

Schneider, K. R., D. S. Wethey, B. S. T. Helmuth, and T. J. Hilbish (2005) Implications of movement behaviour on mussels dislodgment: exogenous selection in a *Mytilus* spp. hybrid zone. Marine Biology 146:333-343.

Seed, R., and R.A. Brown. 1978. Growth as a strategy for survival in two marine bivalves, *Cerastoderma edule* and *Modiolus modiolus*. Journal of Animal Ecology 47:283-292.

Seuront, L. 2013. Complex dynamics in the distribution of players' scoring performance in Rugby Union world cups. Physica A 392:3731-3740.

Seuront, L. 2018. Microplastic leachates impair behavioural vigilance and predator avoidance in a temperate intertidal gastropod. Biology Letters 14:20180453.

Sheldon, K. S., and M. E. Dillon. 2016. Beyond the mean: biological impacts of cryptic temperature change. Integrative and Comparative Biology 56:110-119.

Sih, A., J. Stamps, L. H. Yang, R. McElreath, and M. Ramenofsky. 2010. Behavior as a key component of integrative biology in a human-altered world. Integrative and Comparative Biology 50:934–944.

Simons, N. K., W. W. Weisser, and M. M. Gossner. 2016. Multi-taxa approach shows consistent shifts in arthropod functional traits along grassland land-use intensity gradient. Ecology97:754–764.

Teplitsky C., A. Laurila. 2007. Flexible defense strategies: competition modifies investment in behavioral vs. morphological defenses. Ecology 88:1641-1646.

Van Cauwenberghe, L., M. Claessens, M. B. Vandegehuchte, and C. R. Janssen. 2015. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. Environmental Pollution 199:10-17.

Vered, G., A. Kaplan, D. Avisar, and N. Shenkar, 2019. Using solitary ascidians to assess microplastic and phthalate plasticizers pollution among marine biota: a case study of the eastern Mediterranean and Red Sea. Marine Pollution Bulletin 138:618-625.

Vince, J., and B. D. Hardesty. 2017. Plastic pollution challenges in marine and coastal environments: from local to global gouvernance. Restoration Ecology 25:123-128.

von Moos, N., P. Burkhardt-Holm, and A. Koehler. 2012. Uptake and effects of microplastics on cells and tissues of the blue mussel *Mytilus edulis* L. after experimental exposure. Environmental Science and Technolohy 46:11327-11335.

Wellstein, C., B. Schröder, B. Reineking, and N. E. Zimmermann. 2011. Understanding species and community response to environmental change. A functional trait perspective. Agriculture, Ecosystems & Environment 145:1-4.

Zar, J.H. 1999. Biostatistical analysis, Prentice Hall, Upper Saddle River, NJ.

Zardi, G. I., K. R. Nicastro, C. D. McQuaid, M. Rius, and F. Porri. 2006. Hydrodynamic stress and habitat partitioning between indigenous (*Perna perna*) and invasive (*Mytilus galloprovincialis*) mussels: constraints of an evolutionary strategy. Marine Biology 150:79-88.

TABLE. 1. Synthesis of the evolution of the traits (i.e. aggregation and movement behavior, and byssal thread production) observed in *Mytilus edulis*, *Choromytilus meridionalis*, *Perna perna* and *M. galloprovincialis* held in microplastic leachate seawater; for each trait and each species, the symbols "=" and "+" respectively indicate the absence of significant change or a significant strengthening.

	Aggregation			**Motility**	**Byssal thread production**
	Percentage	Number	Size		
M. edulis	+	=	=	+	=
M. galloprovincialis	=	=	=	=	+
P. perna	=	=	=	=	+
C. meridionalis	+	=	=	+	=

Figure captions

FIG. 1. Temporal patterns of the percentage of aggregation of *Mytilus edulis*, *Choromytilus meridionalis*, *Perna perna* and *M. galloprovincialis* in control seawater (blue) and in microplastic leachate seawater (red). The error bars are the 95% confidence intervals.

FIG. 2. Interspecific comparisons (mean ± 95% confidence interval) among *Mytilus edulis* (Me), *M. galloprovincialis* (Mg), *Perna perna* (Pp) and *Choromytilus meridionalis* (Cm) for the fitting parameters $A_{\max}$ and k describing the temporal patterns of the aggregation rate $A(t)$ as $A(t) = A_{\max}t/(k + t)$ of the experiment for mussels held in control seawater and leachate seawater. The black and open dots respectively indicate the presence and the absence of significant differences in $A_{\max}$ and k between control and microplastic leachate seawater. The horizontal and vertical bars identify groups of measurements that do not differ significantly in control seawater and in leachate seawater, respectively. The dashed line is the first bissectrix, i.e. $A_{\max}(\text{control}) = A_{\max}(\text{leachate})$, and $k(\text{control}) = k(\text{leachate})$.

FIG. 3. Interspecific comparisons (mean ± 95% confidence interval) among *Mytilus edulis* (Me), *M. galloprovincialis* (Mg), *Perna perna* (Pp) and *Choromytilus meridionalis* (Cm) for crawling distance d_c (cm) in the first 3 h of the experiment for mussels held in control seawater, $d_c(\text{control})$, and leachate seawater, $d_c(\text{leachate})$. The black and open dots respectively indicate the presence and the absence of significant differences in d_c between control and microplastic leachate seawater. The dashed line is the first bissectrix, i.e. $d_c(\text{control}) = d_c(\text{leachate})$.

FIG. 4. Interspecific comparisons (mean ± 95% confidence interval) among *Mytilus edulis* (Me), *M. galloprovincialis* (Mg), *Perna perna* (Pp) and *Choromytilus meridionalis* (Cm) for the number of byssal threads produced by mussels held in control seawater, $N(\text{control})$, and

leachate seawater, *N*(leachate). The black and open dots respectively indicate the presence and the absence of significant differences in the number of byssal threads produced between control and microplastic leachate seawater. The horizontal and vertical bars identify groups of measurements that do not differ significantly in control seawater and in leachate seawater, respectively. The dashed line is the first bissectrix, i.e. *N*(control) = *N* (leachate).

611 **Figure 1**

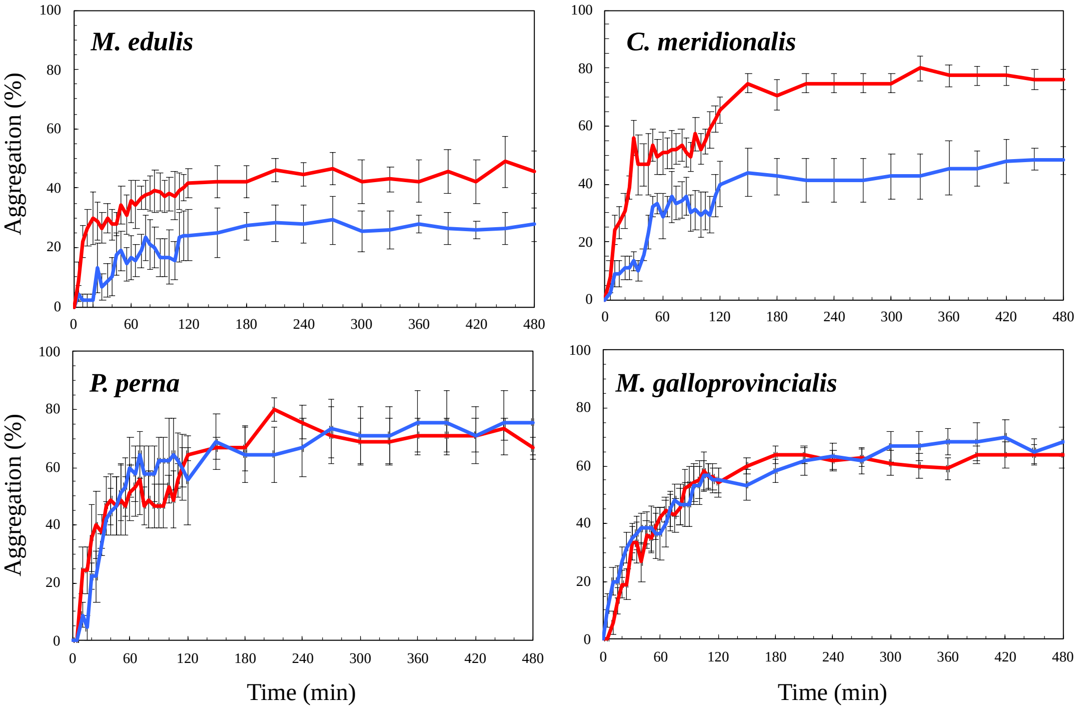

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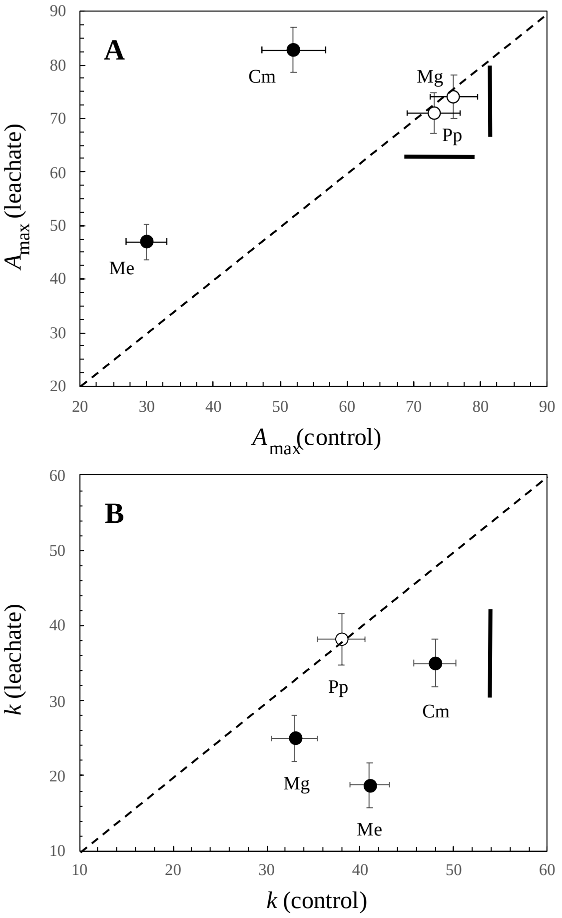
Figure 2


A
Cm
Mg
Pp
Me
A_{max} (leachate)
A_{max}(control)
90
80
70
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80
90
B
Pp
Cm
Mg
Me
k (leachate)
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60
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Figure 3

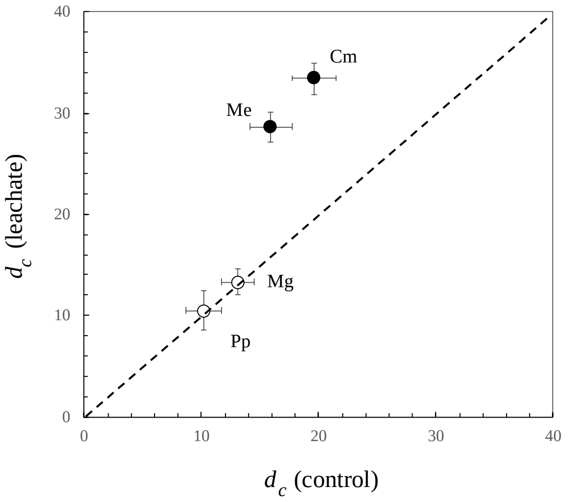

Figure 4