Chapter 20

Design and analysis of laboratory experiments on aquatic plant litter decomposition

Julia Reiss[1], R.A. Bailey[2,3] and Daniel M. Perkins[1]

[1]Department of Life Sciences, Whitelands College, Roehampton University, London SW15 4JD, United Kingdom

[2]School of Mathematical Sciences, Queen Mary University of London, London E1 4NS, United Kingdom;

[3]School of Mathematics and Statistics, University of St Andrews, St Andrews, Fife, KY16 9SS, United Kingdom;

Abstract

Microcosm studies are a useful tool when it comes to studying leaf litter decomposition but designing and analysing them can be a tricky path with many pitfalls. Because there is a plethora of drivers of leaf decomposition, it is important to be precise about the scientific questions that can be addressed with microcosm set-ups, and to use experimental designs that have minimal logistic implications but, at the same time, high statistical power. In this chapter, we first set the scene by introducing a hypothetical study that has the aim to estimate how leaf decomposition is driven by different decomposers and abiotic conditions. Following on from this scenario, we give an overview of the main biotic and abiotic drivers of leaf decomposition that will play a role in laboratory settings (with special attention to consumer identity, species richness, body size and metabolic capacity, and also temperature, time scales and stressors). We then explain how to design and analyse laboratory experiments on aquatic leaf litter decomposition including the mathematics for calculating the metabolic power of leaf decomposers and some statistical models. Finally, three case studies are given (highly controlled experiments that can be analysed by analysis of variance).

Keywords: ANOVA, shredder, microcosm, metabolism

1. Introduction

Leaf litter falling into streams and rivers links energy flow from the terrestrial environments to the aquatic realm and is an important ecosystem process that mobilises large amounts of carbon and other nutrients (Abelho, 2001; Marks, 2019). The extent to which leaves are decomposed, and energy is recycled in aquatic systems, depends on a plethora of abiotic and biotic factors, and these might change and vary with time; and it is often these drivers that are the focus of a microcosm study in the laboratory.

Indeed, laboratory experiments, and using aquatic microcosms, offer a way to disentangle which factors drive leaf decomposition (and if they act in synergy or antagonistically), given they are designed and analysed in a meaningful way. In general, tightly-controlled microcosm experiments offer a window into the complexity of nature (e.g. Bell et al. 2005), and many such studies have been carried out with leaf decomposers (e.g. Jonsson and Malmqvist 2000, Perkins et al. 2010a, Risse-Buhl et al. 2015, Flores et al. 2016, Gonçalves et al. 2019). A typical critique of microcosm studies is that they do not represent nature in a way field studies do; however the latter can often be criticised for not elucidating the mechanisms behind the observed patterns/phenomena. Highly controlled experiments can deliver in this regard (Benton, Solan, Travis, & Sait, 2007) because they can point to the mechanisms, i.e. they can produce the parameters that can be fitted in mathematical models (they inform modelling). Benton et al. (2007) point out how important it is to find these intrinsic mechanisms and how microcosm experiments can short-cut and 'speed up' insights from long-scale field work.

Many research groups have studied leaf litter decomposition in microcosm set-ups (Gonçalves et al., 2019; Pascoal, Cássio, Nikolcheva, & Bärlocher, 2010; Perkins et al., 2015;

Reiss, Bailey, Perkins, Pluchinotta, & Woodward, 2011) because not only is this an important ecosystem process but also it is possible to run experiments with little costs in terms of consumables. Leaf decomposition is further a process that can easily be monitored in microcosms and it is possible to replicate the decomposer communities. In fact, it is even difficult to exclude some leaf consumers such as bacteria and fungi from the laboratory as they 'travel' with the leaves from the field to the laboratory —in some cases even when the leaves are dried.

There is an unavoidable trade-off between replication and realism in all laboratory experiments and it is important to be precise about which questions can be answered in laboratory settings. Further, a central objective of these experiments is that they are planned and analysed in the best possible way and this chapter will give an overview of pitfalls and solutions to studying leaf litter decomposition in the laboratory.

Because this chapter will tackle only some aspects of designing and analysing leaf decomposition experiments, we have highlighted literature that has many relevant references cited within and that will help with further reading (see also Tables 20.1 and 20.2). It is helpful to read this chapter in conjunction with Chapter 21, where a number of approaches have been described for field experiments; and to consult the textbooks 'Methods to study litter decomposition: a practical guide' (Bärlocher, Gessner, & Graça, 2020) and 'Methods in stream ecology' (Hauer & Lamberti, 2007).

There are a number of exciting aspects of leaf decomposition we have not explored in detail in this chapter, such as experimental designs that require 'non-ANOVA' statistical analysis such as generalized additive mixed models (GAMM), explanations of consumer species in detail (Chapters 9, 10 and 11), or food web interactions and implications for decomposition (Chapter 7). We would like to refer the reader to other chapters and

literature when it comes to the general theme of energy transfer from leaves to consumers which includes topics such as the role of nutritional value of leaves for consumers (chapter 3 and (Jabiol, Lecerf, Lamothe, Gessner, & Chauvet, 2019; Larrañaga, Basaguren, & Pozo, 2014), for example by using stoichiometric approaches (Chapter 3; Farrell et al., 2018), a focus on leaf species diversity (Larrañaga et al., 2014; Martínez, Larrañaga, Pérez, Basaguren, & Pozo, 2013), the changes in resource quality over time (Chapter 19; (Cristina Canhoto & Graça, 1996) and switch of consumer assemblage over time (e.g. because of seasonal changes). The focus of this chapter is very much on the consumers rather than the food resources (leaves).

In this chapter, we start with a hypothetical study that has the aim to estimate how biodiversity of consumers drives leaf decomposition to 'set the scene'. Following from this scenario, we give a synthesis of possible drivers of leaf decomposition, followed by an overview of how to design and analyse leaf litter experiments in general. We illustrate the latter by explaining experimental set-ups that have been used in laboratory-based freshwater research and make suggestions for meaningful experimental designs with minimal logistic implications. Finally, we give three case studies to illustrate the importance of meaningful experimental design and analysis when it comes to leaf decomposition experiments. These case studies have in common that they are highly controlled and can be analysed by analysis of variance.

2. Planning leaf decomposition experiments in the laboratory – where to start?

Leaf decomposition is the sum of chemical and physical processes (Chapter 2), and organisms interacting with the leaves, while interacting with each other as well as with their abiotic environment. To approach this complex concept let us imagine an enthusiastic PhD

student. She has identified that the freshwater shrimp *Gammarus* spp. and the freshwater hog louse *Asellus* spp. are abundant macroinvertebrates in the local streams, and are therefore likely to play an important role in leaf decomposition, but she is aware that other invertebrates such as insect larvae can graze on leaves and that tiny crustaceans such as *Cyclops* spp. can also graze on leaf biofilm. She wants to find out which species is the most efficient in terms of breaking leaves down, by feeding on them, and whether a combination of different species results in maximised leaf mass loss. The PhD student has a good understanding of the autecology of freshwater crustaceans, and she knows that within the species individuals vary. For example, the crustaceans will reproduce more than once a year in nature and different sized individuals will be present at different times of the year (i.e., there are small, still growing individuals, as well as males and females). The feeding activity of these individuals is strongly influenced by temperature as they are ectotherms, but other factors such as a suitable habitat play a role; and their growth and reproduction depend on many factors including the quality of their food and water temperature. For her experiments, it will be impossible to separate these crustaceans from bacteria and fungi in the water and these organisms also decompose leaves so she would like to include fungal species in her design.

Obviously, she is faced with a complex jigsaw puzzle if she tries to answer a seemingly simple question. Here we will make an attempt to assemble this puzzle, piece by piece (Figure 20.1) with reference to pivotal literature (see also Tables 20.1 and 20.2), with the knowledge that it will make sense only from a particular point of view (for a selected pool of questions) and an appreciation for the fact that not all pieces of the puzzles are known (Figure 20.1).

Insert figure 20.1 here

3. Biotic and abiotic factors to consider in leaf decomposition experiments

Many biotic and abiotic factors drive leaf decomposition and in the following sections some of them will be discussed in more detail (consumer species identity, species richness, body size and metabolic capacity, and also temperature and time scales; see Table 20.1).

Insert table 20.1 here

3.1. Species identity drives leaf decomposition

A consortium of organisms decomposes leaves in streams and rivers; and bacteria, fungi and animals all play pivotal roles (Hieber & Gessner, 2002). Fungi are an important component of decomposer assemblages associated with plant litter in streams (Gessner et al., 2010) and macro-invertebrates feed on both fungi and leaf material (C. Canhoto & Graça, 2008). The fact that a group of macroinvertebrates has been dubbed 'shredders' indicates that there are species that are specialised in shredding leaf material and indeed we would expect such species to decompose leaves faster and more efficiently than other species. Clearly 'true' leaf litter feeding requires the existence of endogenous cellulases (fungi and shredders) or exocellulases (free living bacteria and endosymbiotic bacteria) (Zimmer, 2005). For example, snails might graze on leaf biofilm and indirectly aid the decomposition of leaves but they do not feed on as much leaf material as *Gammarus* which is a leaf shredding amphipod that has, just like the freshwater hog louse *Asellus,* endosymbiotic bacteria in its gut that can break down cellulose (Zimmer, 2006) (however – a word of caution: *Gammarus* and *Asellus* are strictly speaking omnivorous). Species identity, or at

least a specific combination of traits, can therefore be an important driver of leaf decomposition - although surprisingly few studies have tested this. For example, the larvae of the cased caddisfly *Sericostoma* are very efficient in shredding leaves fast (e.g. González and Graça 2003, Reiss et al. 2011), which is possibly due to its large size and high metabolic demands (see section 3.2.), but, of course, it is conceivable that they also possess very efficient cellulases, or gut symbionts.

3.2. Body size, biomass and metabolic rate drive ecosystem processes: calculating metabolic capacity

Earth's biota regulates numerous fluxes of energy and matter, including carbon uptake, nutrient cycling and oxygen production. When measured at local scales, these rates are referred to as 'ecosystem processes' and leaf decomposition is such a process. The activity of all organisms on earth is constrained by the laws of physics and chemistry and this simple fact can be of immense help when studying ecosystem processes such as leaf decomposition because all organisms can operate only within the (mathematical!) limits of natural laws. This becomes apparent when studying metabolism and how this relates to the size of an organism, and, in turn, to how the organism can contribute towards ecosystem processes. Body size and metabolism are a hot topic in Ecology (Whitfield, 2004) because metabolism 'sets the pace of life' and drives processes across levels of biological organisation (from individuals to ecosystems) (Brown et al. 2004). As a consequence, body mass is clearly a trait that needs to be considered when we study processes driven by organisms because body mass determines the basal metabolic rate, energy demands and ingestion rates of an individual (Brown et al., 2004; Perkins, McKie, et al., 2010; Peters, 1983; Reiss, Bridle, Montoya, & Woodward, 2009; Woodward et al., 2005). It follows that

the performance of an assemblage is the sum of the metabolic power of its constituent individuals; or phrased in a different way; the body-mass vs. biomass distribution in that assemblage. It is hence not surprising that there is a growing number of studies starting to consider the role of body size and biomass in experiments that address ecosystem process rates (Flores et al., 2016; McKie et al., 2008; Perkins, McKie, et al., 2010; Reiss, Bailey, Cássio, Woodward, & Pascoal, 2010; Reiss et al., 2011). Species identity is often confounded with body mass and using many differently sized individuals within a species or among species circumvents this problem and allows distinguishing between taxonomic and functional diversity and developing a more general mechanistic and predictive framework. Hence, we argue here that many future studies on leaf decomposition would profit from taking the body mass distribution of the leaf consumers into account and we encourage a more theoretical approach to leaf decomposition.

Because the activity of organisms can be understood through physical laws, such as laws of mass and energy balance, and thermodynamics, it is possible to use parameters such as body size and temperature (see section 3.3) to predict how effective an assemblage of individuals will be when it comes to decomposition of leaf litter. A potentially promising approach to link the effects of body mass and temperature on ecosystem processes is through the application of the "Metabolic Theory of Ecology" (MTE) (*sensu* Brown et al. 2004). Building upon well-established body size allometries (Peters, 1983) and temperature scaling based upon first order physical principles (Gillooly, Brown, West, Savage, & Charnov, 2001), the MTE can be used to make quantitative predictions about a wide range of biological processes across levels of organisation (Brown et al., 2004). Leaf decomposition is the amalgamation of leaf feeding by different consumers. The feeding rate of each consumer species is, in turn, strongly connected to the body mass of individuals. If the body

size distribution and abundance of leaf decomposers is known then it is possible to calculate metabolic rates (which correlate strongly with leaf decomposition) of individuals (Table 20.2) and those of entire assemblages (the so called 'metabolic capacity' (*sensu* Ruesink and Srivastava (2001); also called 'metabolic potential', see Table 20.2).

Insert table 20.2 here

To summarise the above, given that allometric scaling relationship between individual body mass and metabolism is less than unity (Brown et al., 2004), the spectrum of individual body sizes characterizing a given assemblage is important in determining process rates. In the following sections we will shortly dive into more details about one important aspect of this, which is not necessarily intuitive. While an assemblage composed of larger individuals may have a high total metabolic capacity, an assemblage composed of small individuals will exhibit higher mass-specific process rates (Peters, 1983). To illustrate the latter, let us imagine a hypothetical experiment with two crustaceans: *Asellus* and *Cyclops*. Individuals of *Asellus* are much larger than individuals of *Cyclops* but their metabolic rate per unit body mass is lower. This means that, if these two species are used in a laboratory setting, then *Cyclops* assemblages will consume more food over time compared to *Asellus* - given the biomass of the two assemblages is the same. Hence, small animals ingest more food in relation to their own body mass compared to larger organisms. Applying this knowledge to experimental microcosm set-ups means it is extremely important to calculate the metabolic capacity (Table 20.2). Indeed, instead of adjusting for number or biomass (as done in most leaf decomposition experiments), the 'metabolic capacity' should be calculated (see Table 20.2). Metabolic capacity is a proxy for how much energy a given assemblage will use and

how fast it will use it. In terms of adding individuals to microcosms, adjusting for metabolic capacity will always result in a lower number of small individuals (compared to larger individuals) than adjusting for the biomass, as the following example explains. Picture two microcosms, one for an *Asellus* only treatment and one for a *Cyclops* only treatment; and the aim is to control for body mass differences in these assemblages. Assuming we use very small *Asellus* individuals that are 6.6mm long on average (~2.14mg dry weight [DW]) means they weigh almost 71 times more than *Cyclops* (~1.6mm long, 0.03mg DW). Hence, when biomass is accounted for, 71 more *Cyclops* individuals are added for each *Asellus* individual. However, adjusting for metabolic capacity means that only 18 times more *Cyclops* are added for one *Asellus* individual (see Table 20.2 and for an example see Flores et al., 2016). Metabolic capacity can be a strong predictor of leaf decomposition (Figure 20.2) and can also be extended to incorporating the effects of temperature (Figure 20.2), as explained towards the end of 3.3.

Insert figure 20.2 here

3.3. *Temperature affects leaf decomposition*

Leaf mass loss in fresh water is strongly modulated by temperature, and a simple explanation is that the leaves 'leach' various substances when exposed to water, including phenols (e.g. tannins) (Bärlocher, 2005; Cristina Canhoto & Graça, 1996; Quinn, Burrell, & Parkyn, 2000). Leaching will follow chemical- and temperature rules and is unavoidable. Other abiotic factors such as light surely also play a role here and studies that manipulate light intensity in the laboratory must make sure that light intensity and temperature are not confounded.

Temperature also affects leaf decomposers, either over longer time scales (evolutionary response of organisms) or in a very direct, immediate way. Regarding the first point, numerous recent studies from fresh waters have suggested that environmental warming often favours smaller organisms, both within and among species, and that much of this can be ascribed to metabolic and physiological constraints that scale allometrically with body mass (see review by Perkins et al. 2010b, and references therein). A high level of genetic variation that allows a species to adapt (Gamfeldt, Wallén, Jonsson, Berntsson, & Havenhand, 2005) is therefore an advantage when it comes to changes in the environment such as temperature regimes. Hence, evolutionary response to temperature should be considered in laboratory experiments that are run over time periods in which organisms reproduce and potentially evolve (e.g. in most lab experiments bacteria will evolve within hours).

Critically for all types of laboratory experiments, temperature affects organisms in a very immediate fashion, through increasing energetic demands of ectotherms that have no choice but to increase their metabolism with temperature. For instance, rates of consumer ingestion and resource depletion increase exponentially with temperature, and as a rule of thumb, physiological rates approximately double with a 10°C increase in temperature (the so called Q_{10} rule). Consequently, important new insights have been gained through integrating measures of the metabolic capacity of consumers (Flores et al., 2016; Ruesink & Srivastava, 2001) and temperature scaling (Gillooly et al., 2001) into laboratory studies (Perkins et al., 2015, 2012). Again, the MTE offers the mathematical models behind temperature effects and much of this is based on the Boltzmann constant and Arrhenius equation (Gillooly et al. 2001; Table 20.2). For example, it is possible to predict a rate, such as leaf decomposition, for a hypothetical temperature, T_2, if the rate is known for a

reference temperature, T_1 (Gillooly et al., 2001). Further, it is possible to incorporate the effects of temperature (in addition to body size) on metabolic capacity (Table 20.2, Figure 20.2), and in doing so this approach can be extended to predict 'assemblage metabolism' (Table 20.2, Figure 20.2). For instance, it is useful to calculate assemblage metabolism if leaf decomposition is run at different temperatures and the results from these temperatures are merged (see example in Figure 20.2).

3.4. Biodiversity and species interactions drive leaf decomposition

Metabolic power of decomposer assemblages and temperature are clearly strong predictors for plant litter decomposition but past litter decomposition experiments often had a strong focus on biodiversity effects (Perkins, McKie, et al., 2010; Reiss et al., 2010, 2011). The general premise here is that assemblages that are very biodiverse will drive processes such as leaf litter decomposition better (e.g. faster) than assemblages that are less biodiverse. Biodiversity can be measured in different ways, with species richness being the most popular metric, and has indeed been shown to increase many ecosystem processes (see review by Reiss et al. 2009). When litter decomposition driven by a decomposer assemblage is measured a key question is: do species-rich assemblages exhibit faster rates than species-poor ones? There are three possible scenarios here: the different species within that assemblage can 1) perform in an additive fashion, i.e. as they would alone ('in monoculture'), 2) interact with each other and influence each other in a positive or negative way and 3) drive different processes that contribute to an overall effect (multifunctionality). In laboratory studies where species are offered a limited amount of food resource types and environmental conditions, they often perform in an additive fashion — meaning once metabolic capacity is accounted for, species decompose the same amount of leaf litter

(Flores et al., 2016; Reiss et al., 2010, 2011). True biodiversity effects often only become apparent when a 'multifunctionality approach' is considered because species contribute to more than one process and respond differently to interactions with biotic and abiotic factors (Gamfeldt, Hillebrand, & Jonsson, 2008; Gamfeldt & Roger, 2017; Perkins et al., 2015). In the context of a leaf decomposition laboratory experiment this means that on top of measuring leaf decomposition, measuring other processes (e.g. production of faeces, respiration rates) will provide a more complete picture of the importance of biodiversity (Gamfeldt et al., 2008; Reiss et al., 2009).

So far we have focused on biodiversity within a single trophic level but of course leaf consumers are part of complex trophic interactions in fresh water ecosystems (Gessner et al., 2010; Reiss et al., 2009). While studying the macrofauna-fungi-leaf relationship has a longer tradition in laboratory experiments (e.g. Canhoto and Graça 2008, Reiss et al. 2010), laboratory studies that involve microscopic consumers (such as protozoans and micro-metazoans) and leaf bacteria and fungi are on the rise (Chambord, Tackx, Chauvet, Escolar, & Colas, 2017; Flores et al., 2016; Ribblett, Palmer, & Wayne Coats, 2005; Risse-Buhl et al., 2012); and they have shown that tropic interactions among these small organisms can have a substantial impact on leaf decomposition (e.g. Risse-Buhl et al. 2015, Chambord et al. 2017).

3.5. Other abiotic factors and stressors

Species interactions and performance are influenced by abiotic factors other than temperature, of course, and an example is habitat complexity. For example, it is conceivable that different consumer species feeding on the same food resource interact less when habitat complexity is high or that complexity influences the overall performance of an

assemblage because species can operate in their 'optimal' dimensional environment. However, to our knowledge, there are very few studies testing this hypothesis (Flores et al., 2016), but there is strong evidence that species are adapted (foraging and feeding) to the dimensionality of their environment (Pawar, Dell, & Savage, 2012).

Most of the world's fresh waters are affected by global change and freshwater organisms are faced with a range of environmental changes such as pollution (including acidification), anoxia or light penetration (Ormerod, Dobson, Hildrew, & Townsend, 2010). Leaf decomposition is clearly an ecosystem process that is heavily influenced by environmental change (see chapters 12-18) and laboratory experiments offer a way to target these issues (Cristina Canhoto, Simões, Gonçalves, Guilhermino, & Bärlocher, 2017; Gonçalves et al., 2019; Medeiros, Pascoal, & Graça, 2009). To complicate matters, time scales are extremely important when it comes to estimating the effects of stressors because most species can endure non-optimal conditions for short periods, but not over longer (reproductive) time scales.

3.6. It gets complicated: a more realistic picture of what drives leaf decomposition

We have expanded on only a few drivers of leaf decomposition here and, of course, are faced with the fact that they all play a role in this ecosystem process. For the purpose of a laboratory experiment, the questions must be very precise because it is rather obvious that it is extremely difficult to take all drivers into account. Drivers such as species identity, biomass or temperature can be confounded and influence each other. Moreover, leaf decomposition in nature is a very dynamic process where one driver might be important at one point in time but have negligible effects at some other. For example, leaf consumers will reproduce over time and generation time depends on body size and temperature. Further,

reproduction will depend on the nutritional quality and the quantity of leaf material and the presence of other food resources. Clearly, in any case, leaf decomposition is strongly driven by the biomass and metabolic capacity of the consumers and possibly also driven by biodiversity and interactions between the consumers. Still, laboratory experiments can distil some of the mechanisms that operate in nature and we can ask meaningful questions that can be addressed with appropriate statistical analysis as described in the following section.

4. Statistical approaches: maximising statistical power while reducing logistics

In the following sections, some general approaches will be described that help to maximise statistical power and to minimise logistics when drawing up an experimental design for a laboratory study with leaf litter and decomposers. The research question will determine the experimental design for every experiment; hence we give examples for possible questions while explaining statistical approaches.

4.1 Analysis of variance

In laboratory experiments as described here, the objective is to test which predictors describe a certain response best. In other words, we want to find out which factors contribute to leaf mass loss. Let us imagine a more complex experiment in which seven species are used (Figure 20.3). The aim is to find out if species richness (e.g. 1, 2 and 3 species feeding on leaves together) or species identity (e.g. *Asellus*, *Gammarus*, etc.; see Figure 20.3a) are responsible for leaf decomposition. In the example just given, there are 63 possible combinations or 'treatments' if all seven species are run as mono, di- and tri-cultures and, of course, they will have to be run in replication (e.g. let us assume 2 replicates, so 126 microcosms). These treatments represent 63 different 'assemblage

identities'. Assemblage identity can be fitted as a predictor of leaf decomposition but really we are interested in whether species richness has an effect on the response or if species have particular effects. Assemblage identity and species richness represent mathematical 'models' that can be fitted in a statistical test. The overall aim is to compare means (of the replicates) for each level within a predictor (e.g. the predictor 'species richness' or 'assemblage identity') and to find out which predictor (or combination of predictors) describes the response best in terms of low variance around those means. For these aims and assumptions, a *t*-test would be the analysis of choice if we only compare two means but for more complex questions, as in our example, analysis of variance (ANOVA) can be an appropriate and popular tool when it comes to data analysis.

Insert figure 20.3 here

4.2 Running designs that are not fully factorial – statistical power and logistics

Ideally laboratory experiments are run in a fully factorial design because these deliver optimal statistical power (if combined with high replication). Clearly this is not always possible, especially when larger organisms are used that are laborious to sample in the field, and when laboratory space is limited. In this case, it is important to not randomly run certain treatments but to instead make sure that statistical power is maintained. For example, let us consider, again, the example of seven species (Figure 20.3) in a leaf decomposition experiment with a biodiversity focus (see Reiss et al. (2010) for example). There are 35 possible tri-cultures from 7 species, making the set-up rather large with 63 treatments (mono, di- and tri-cultures). We want to keep the experiment manageable in size while obtaining clear information about the effect of each species on each other

species. Rather than using a random collection of tri-cultures, statistical power is higher if each pair of species occurs together exactly once (Figure 20.3) and this gives 7 tri-cultures instead of 35 (Figure 20.3 and see Reiss et al., 2010). Those can be randomly assigned to replicated microcosms, or — even better — be run in blocks (see section 4.5).

4.3 'Visualising ANOVA' – Hasse diagrams

ANOVA cannot only be used when a predictor has more than two levels (e.g. the predictor 'species richness' has levels 1, 2 and 3) but more than one predictor (or 'model') can be fitted in a family of models (Bailey 2008). For instance, we can fit both species richness and assemblage identity as predictors in the same analysis (Figure 20.3). It should be noted that many ecologists will call a collection of models 'ANOVA model' and a single model 'independent variable' and we would like to point out that we are using terminology popular among statisticians (i.e. the term 'model' and 'predictor' instead of 'factor' or 'independent variable'). By fitting ANOVA, we can take into account that some predictors are related (they can be sub-sets of each other — they are 'nested', as in our example of assemblage identity and species richness) and that sometimes the response (leaf decomposition) is best explained by more than one model or even by an 'interaction' of models. The latter would tell us that a certain combination of predictors must be present to drive leaf mass loss best.

Bailey (2008, 2020) recommends showing the family of considered models in a Hasse diagram (see Figure 20.3 and Reiss et al. 2010, Bailey and Reiss 2014, and Bailey 2020 for examples). In these diagrams, there is one dot for each model and it is useful to show the dimension of each model as well as its name. The diagram also contains edges linking some dots. The convention is that if model M1 contains model M2 then the dot for M1 is higher

than the dot for M2 and there is a chain of generally downward edges linking the dot for M1 to the dot for M2 (see example in Figure 20.3d).

4.4 ***Fitting statistical models in ANOVA that can disentangle additive vs facilitation or antagonistic effects***

A typical question behind B-EF research is: do species-rich communities drive ecosystem processes better than species-poor ones? To test this in laboratory experiments, we must be able to find out what a species does in isolation, what it does in combination with another species and if any interactions between the species are antagonistic, additive or if the species even facilitate each other. This can be done by using a fully factorial experimental design (but see section 4.2.) and the appropriate ANOVA models. One such model dubbed 'Type' (Bailey & Reiss, 2014; Reiss et al., 2010, 2011) is so called because it focusses on the effects of different 'types' rather than species per se. We could have called it 'species identity' but in some instances we want to use individuals of the same species that differ in terms of their traits (e.g. body mass). For example, within one species we could distinguish small and large individuals – two types. This 'type' model assumes that each type has a unique effect, which provokes a characteristic response irrespective of whether the type is combined with other types or not. For example, Reiss et al. (2011) used small and large individuals of the water hog louse *Asellus* in leaf decomposition experiments (in combination with other shredders in mono, di and tri-cultures). In this experiment, metabolic capacity was not accounted for, individuals were simply added in equal numbers when they were in polyculture (e.g. halved numbers in di-culture). The 'type' model that was fitted in the ANOVA therefore had a simple rationale (it was assumed that the response simply depends on additive effects of types) that can be illustrated with the following

example. If 12 small *Asellus* feed on 0.6g of leaf material over 28 days and 12 large *Asellus* feed on 0.7g under the same conditions, then a di-culture of the two 'types' should feed on 0.65 g ((0.7+0.6)/2) if the numbers of each type are half in di-culture (that contains 6 small *Asellus* and 6 large *Asellus*). The response here is leaf mass loss but, of course, it can be replaced with other responses measured (such as algal grazing, amount of faeces produced etc.; see Perkins et al. 2015). Hence, the ANOVA essentially tests if the di-culture does indeed feed on 0.65g.

In terms of the statistical model fitted, the response on monoculture A (the small *Asellus* monoculture) should be α_1; the response on monoculture B (the large *Asellus* monoculture) should be α_2; the response on di-culture AB should be $(\alpha_1+ \alpha_2)/2$. If we imagine a third type, such as small *Gammarus* (monoculture 'C'), and a tri-culture with 4 individuals in it of each type, the response on tri-culture ABC should be $(\alpha_1 + \alpha_2 + \alpha_3)/3$, and so forth. If this model, considered in the hierarchy of other models, explains the response best (and has the best AIC if calculated), we might conclude that there are no biodiversity effects (and no species interactions) on the particular response, under the lab conditions. However, a word of caution here: despite being counter-intuitive at first glance, although this model concludes that there are no species richness effects, this model can be significant in cases where biodiversity is indeed important for combined processes. This will be the case when multifunctionality (i.e. more than one process) is measured (Perkins et al., 2015).

4.5 ***Replication, blocks, randomisation and pseudoreplication***

When the experimental set up is so large that not all microcosms can fit into the same location at the same time (in a randomised arrangement) then high statistical power is maintained if sub-sets of the experiment are run in blocks where, generally speaking, it is

the replicates that are run in blocks (e.g. one replicate per treatment in each block). These blocks can be rooms, shelves, or, most often, time. Block effects can then be accounted for in the ANOVA because 'block' can be fitted as a model in the analysis (see Reiss et al. 2010, 2011 for examples).

Pseudoreplication (also called false replication) can occur when replicates are confounded with another variable that might have an influence on the response (Bailey & Greenwood, 2018; Hurlbert, 1984; Johnstone, 2013), such as a temperature cabinet or a location (e.g. a section of a river or a room). For example, Perkins et al. (2015) tested how leaf mass loss changed across three temperatures but temperature was not replicated as such because each temperature was confined to a particular temperature cabinet. In order to replicate temperature, more than one temperature cabinet should be run at the same time (e.g. two cabinets with the same temperature) — or a carefully planned block design should be used. For example, if there are three temperature cabinets that are set to three different temperatures, then it is possible to run the experiment with only some replicates at those three temperatures and to then repeat it at a later point, this time running the remaining replicates and switching cabinets (e.g. the cabinet that was used for 10°C is set to 15°C and so forth). In the analysis, time can be fitted as a block (block 1 is the first run, block 2 is the second run and so forth). Cabinets are fitted as sub-blocks with random effects. To test whether the predictor `temperature' is needed in the model, the differences between temperatures are compared with the differences between cabinets within blocks.

5 Examples of laboratory experiments on aquatic leaf litter decomposition

In this chapter, we aim to convey that laboratory experiments on leaf decomposition are a popular tool for freshwater ecologists because they are logistically feasible, affordable ways

to approach (rather theoretical) questions in ecology. In the following we will illustrate our earlier thoughts about drivers of leaf decomposition, experimental design and analysis by giving three examples for laboratory studies that measured leaf decomposition and which highlight the opportunities these approaches offer to ecologists.

5.1 Flores et al. 2016 – effects of biodiversity, species identity and habitat complexity on leaf decomposition

One such experiment is by Flores and colleagues (Flores et al., 2016) and it had two main questions: does habitat complexity have an effect on leaf decomposition and can a species-rich assemblage of macro-shredders drive leaf decomposition better than a single species? The authors knew that habitat complexity can provide refuge for prey and hence lower predation rates and they hypothesised that other species interactions and therefore ecosystem processes might be connected to habitat structure. They manipulated habitat complexity using different configurations of plastic plants that they added to aquatic microcosms containing decomposing alder leaves. Three different detritivores species (*Asellus*, *Gammarus* and *Cyclops*) were subsequently introduced to these microcosms in a fashion that 3 levels of species richness were created (monocultures and all possible di- and tri-cultures). A focus here was to measure habitat complexity as fractal dimension but creating 4 levels of fractal dimension (including a treatment without plastic plants) meant that other predictors were confounded with habitat complexity and had to be accounted for. Rather than fitting 'habitat complexity' as a predictor, a more precise approach was taken by distinguishing: structure present vs. structure absent, amount (i.e. mass) of structures and fractal dimension of structures.

Another predictor was 'species richness' and to complicate matters, three response variables were measured: leaf mass loss, production of FPOM and water pH. A seemingly simple experiment hence included complex assumptions about the traits of the species used and a rather intricate experimental design, that was not fully factorial, yet logistically feasible for the ecologists and optimised in terms of the analysis (admittedly with the help of a statistician, R.A. Bailey).

In previous experiments, they had found that two of these species, *Asellus* and *Gammarus*, did not interact when they were in combination with each other and hence a third crustacean, *Cyclops* was used. *Cyclops* is much smaller than the other two species and should feed in a different fashion (on leaf biofilm and faeces) and they also perceive structure differently. Importantly, in these experiments the number of crustaceans used was determined by calculating the metabolic capacity for mini-assemblages of these three species. Because small animals ingest more food in relation to their own body mass compared to larger organisms (see section 3.2), adjusting for the metabolic capacity resulted in a smaller proportion of *Cyclops* vs. *Asellus* individuals than adjusting for the biomass. In this case, this meant that in monocultures, 218 individuals were added to the '*Cyclops* only' treatments to give the same metabolic power as 15 individuals of *Gammarus* or 12 *Asellus* individuals in these respective monocultures. Half of these numbers were used when species were combined in di-cultures (e.g. 109 *Cyclops* individuals and 6 *Asellus* were present in the *Cyclops*/*Asellus* di-culture) and tri-cultures contained ⅓ of the monoculture individuals. This meant that all microcosms (potentially) had the same metabolic power.

With this set-up, it was possible to address two questions: 1) do processes associated with leaf decomposition increase with increasing complexity? and 2) is habitat complexity more important for processes than species interactions or identity *per se*? The rationale

here was that more complex environments generally enhance foraging and feeding, and that polycultures would probably not perform in an unexpected way. Overall, the purpose was to show that habitat complexity influences plant litter decomposition and indeed the experiment provided some proof for this — two out of the three processes were linked to complexity. Microcosms with artificial plants in them had more FPOM and lower pH compared to microcosms without these artificial structures. The authors hypothesised that this could be caused by higher digestion and respiration when structures were present. Only taking the microcosms with artificial plants into account, it became obvious that the amount of structure (i.e. amount of plastic plant added) was a stronger predictor of the response variables than the fractal dimension of the structures.

The experimental design and analysis of this experiment is potentially a good template for experiments that are designed to elucidate how stressors affect leaf decomposition. Although 'complexity' was not a stressor, it is an abiotic factor that can be replaced by another one (such as 'light' or 'micropollutant') in terms of experimental design.

5.2 Reiss et al. 2010 – effects of biodiversity on leaf decomposition

Reiss and colleagues (2010) designed and ran an experiment to address effects of biodiversity of fungi and shredders on leaf decomposition. The rationale here was that a large body of research has revealed (often) positive B-EF relationships in manipulative experiments. The vast majority of such studies have focused on either micro- or macro-organisms, but this was the first study to manipulate the diversity of both simultaneously under controlled laboratory conditions. Reiss et al. (2010) performed a microcosm experiment in which they manipulated species richness of aquatic fungi and invertebrates, two taxonomically distant sets of consumers that contribute to the same key ecosystem

process in fresh waters, the decomposition of terrestrial leaf-litter. They used a statistical design to maximize parsimony and analytical power in an experiment with three levels of species richness (7 monoculture, 21 di-culture, and 7 tri-culture treatments). Litter decomposition was measured as both mass loss and the production of fine particulate organic matter (FPOM). They tested whether species richness affected these two processes or whether polycultures performed as predicted from their component monocultures. Further, they calculated assemblage metabolism in each microcosm to test whether the processes were driven by the metabolic demands of fungi and invertebrates.

In general, across the 35 treatments, most species performed in an additive fashion and there was no effect of species richness on either process. There was evidence of assemblage identity effects (i.e. certain species combinations not performing as expected), with instances of significant differences for species combinations that contained both caddisfly larvae and fungi. These assemblages performed worse than expected, which might have been due to dual vertical and horizontal interactions, with the possibility that although both consumed litter directly the former may also have grazed on the latter. Apart from these particular species combinations, overall performance of a species in polyculture was effectively the same as in monoculture and reflected its metabolic demands. This suggests that even taxonomically distant consumers might exhibit a degree of functional redundancy for certain processes provided the remaining species can attain sufficient population biomass (and hence metabolic capacity) to compensate for the loss of other species, although whether such compensatory mechanisms operate in the field remains unknown. Further, species contribute to a multitude of ecosystem processes and progressively more species are needed to sustain the sum of them (Gamfeldt & Roger, 2017). This experiment highlighted how important it is to take metabolic demands into account and demonstrated

the dominance of additive effects of leaf decomposers (demonstrated by the good fit of the 'type' statistical model that was fitted in the ANOVA, see Figure 20.4).

Insert figure 20.4 here

5.3 Perkins et al. 2015 – species contribute to more than one ecosystem process (multifunctionality)

The laboratory experiments by Perkins et al. (2015) were an extension of the B-EF focus by Reiss et al. (2010). Reiss et al. (2010) had found evidence for redundancy among leaf consumers, i.e. if a certain leaf decomposer was absent it could be compensated for by another. Species redundancy has been suggested as a widespread insurance mechanism against the effects of biodiversity loss on ecosystem functioning in the face of environmental change. Redundancy may be compromised when multiple ecosystem processes (termed multifunctionality) and environmental contexts are considered, yet very few studies have quantified this explicitly to date. Perkins et al. (2015) measured five key processes and their combined multifunctionality at three temperatures (5°C, 10°C and 15°C) in freshwater microcosms containing different animal assemblages (1-4 benthic macro-invertebrate species). For single processes, biodiversity effects were weak and were best predicted by additive-based models i.e. polyculture performances represented the sum of their monoculture parts (see Figures 20.2 and 20.4) and this echoed the results of Reiss et al. (2010) (Figure 20.4). Indeed, if individuals are added to microcosms without adjusting for metabolic capacity, the 'type model' (see section 4.4) will describe leaf decomposition best (Figure 20.4) highlighting how important it is to fit this statistical model in the ANOVA and the additive effects of species. Yet, it makes little sense to fit the model if metabolic capacity

is taken into account because effects that are due to species having different body sizes is removed (Figure 20.4; (Flores et al., 2016).

Perkins et al. (2015) did not account for metabolic capacity, however, and concluded that the performance of species was additive when they considered one process alone (e.g. leaf decomposition, Figure 20.4). There were, however, significant effects of biodiversity on multifunctionality (all 5 processes are evaluated combined) at the low and high (but not intermediate) temperature. Variation in the contribution of species to processes across temperatures meant that multifunctionality was promoted by multiple species: greater biodiversity was required to sustain multifunctionality across different temperatures than was the case for single processes or temperatures. This suggests that previous studies may have overestimated the scope for redundancy to buffer the consequences of biodiversity loss in a changing environment. Certainly, this experiment shows that temperature affects leaf decomposers in predictable ways (metabolic scaling laws) and illustrates how vital it is to record temperature in leaf decomposition studies.

6 Conclusions

Let us revisit the keen PhD student who set out to explore the effects of different leaf decomposers, and abiotic factors, on leaf mass loss. In this chapter, we have shown that we have the theoretical background (metabolic scaling laws), and the statistical frameworks, to plan and analyse meaningful leaf decomposition experiments. The PhD student scenario gave the example of an eager researcher who is faced with a plethora of factors that drive leaf decomposition and, by extension, potentially challenging experimental designs. Here, we showed that, in many instances, laboratory experiments can address precise questions when the metabolic demands of the decomposers are considered. For example, in our first

case study, Flores and colleagues (2016) used metabolic theory to calculate metabolic capacity of the mini-assemblages they added to their aquatic microcosms (see Flores et al. 2016 and section 5.1.), which gave them the tools to disentangle species identity effects from body mass effects. Further, we showed that it is possible to streamline laboratory set-ups by making detailed plans about the statistical analysis. The overall aim here is to establish that the analysis can address the questions while minimising logistics.

For example, Flores et al. (2016) did not have to run a fully factorial design but the set-up still retained high statistical power. Hence it was possible for the experimenters to run fewer microcosms, and this allowed them to expand on questions and aims, as well as to include the effects of an abiotic driver (habitat complexity). In summary, in this chapter, we show that the experimental design and analysis will depend on which drivers of leaf decomposition are considered, the scientific questions and on whether the set-up in the laboratory can yield robust data. Because organisms can operate only within the restraints of natural laws, we can use theoretical frameworks to refine how we plan laboratory studies on leaf mass loss.

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Table 20.1 Drivers of leaf decomposition discussed in this chapter that should be considered in laboratory experiments with leaf decomposers

Drivers of leaf decomposition	Explanation	Examples
Species identity	Species vary in their contribution to leaf decomposition and in terms of their preferences (Bärlocher et al., 2020; Tachet, Richoux, Bournaud, & Usseglio-Polatera, 2010).	(Reiss et al., 2011; Swan & Palmer, 2006; Treton, Chauvet, & Charcosset, 2004)
Biodiversity	If different species, different genotypes or different functional groups (and other entities of 'biodiversity') influence each other's performances, or contribute to different pathways in leaf decomposition, then biodiversity influences this ecosystem process (Reiss et al., 2009).	(Andrade, Pascoal, & Cássio, 2016; Flores et al., 2016; Perkins et al., 2015; Reiss et al., 2010, 2011)
Interactions between leaf decomposers	All individuals within an ecosystem are connected via vertical and horizontal linkages, for example within the food web. For instance, feeding interactions (trophic cascades) can alter leaf decomposition rates (Reiss et al., 2009).	(Chambord et al., 2017; Duarte, Pascoal, Cássio, & Bärlocher, 2006; Foucreau, Puijalon, Hervant, & Piscart, 2013; Mille-Lindblom, Fischer, & J. Tranvik, 2006; Ribblett et al., 2005; Risse-Buhl et al., 2012; Risse-Buhl et al., 2013; Treton et al., 2004)
Body mass and metabolic rate	The performance of a leaf decomposer will depend on how large the organism is because large organisms have higher metabolic rates compared to smaller ones (Brown et al., 2004).	(Flores et al., 2016; Reiss et al., 2011)
Biomass	It is important to know whether total biomass is composed of small or large individuals, because the former will exhibit higher process rates for a given unit of mass (small organisms have a higher metabolic rate per unit of body mass) (Brown et al., 2004).	(Reiss et al., 2011)
Metabolic capacity	The metabolic capacity of an assemblage is its potential to contribute towards a certain process (e.g. to decompose leaves) (Brown et al., 2004; Gillooly et al., 2001; Peters, 1983).	(Flores et al., 2016; Perkins, McKie, et al., 2010)
Temperature - metabolic capacity	The metabolic capacity of an assemblage increases with temperature until the organisms surpass their temperature optimum (Gillooly et al., 2001).	(Martínez, Larrañaga, Pérez, Descals, & Pozo, 2014; Perkins et al., 2015; Perkins, Reiss, et al., 2010)
Temperature and time – reproductive potential of consumers	Generation time (and other related characteristics such as fungal sporulation rate) increases with temperature until the species' temperature optimum is reached (Gillooly et al., 2001; Reiss & Schmid-Araya, 2010).	(Martínez et al., 2014)
Temperature - chemical processes	The higher the temperature, the quicker the chemical reactions, such as leaching of phenols from the leaves (Abelho, 2001; Bärlocher, 2005).	We are not aware of laboratory studies testing a range of temperatures in the absence of organisms.
Effects of time and effects of chemical processes	Leaf decomposition changes over time (e.g. because consumer composition changes [Abelho 2001]). In a laboratory set-up it has been shown how leaching changes over time (France, Culbert, Freeborough, & Peters, 1997).	(France et al., 1997)
Stressors	Stressors such as water pollution (Ormerod et al., 2010) can affect leaf decomposers and hence decomposition. Further, they can have direct, chemical effects (e.g. on water pH).	(Cristina Canhoto et al., 2017; Gonçalves et al., 2019; Pascoal & Cássio, 2004)
Other	Other factors influence leaf decomposition. Examples are: habitat complexity (modulates interactions); nutritional value of leaves, water chemistry (e.g. oxygen concentration) or	(Flores et al., 2016; Larrañaga et al., 2014; Risse-Buhl et al., 2013)

	water current (sheer stress)	

Table 20.2: Body mass as a driver of leaf decomposition and ways to measure the impact of this predictor

Calculation for...	**Equation**	**Abbreviations**	**Worked example for an assemblage of 5 individuals of *Asellus aquaticus* (all 10mm long and 2.5mm wide), at 15 ºC**
Body mass (M) of one individual	The equation is species-specific. If the equation is unknown, the volume (V) of the individual can be calculated from a geometric shape (e.g. a spheroid) and V can be converted to mass by knowing the density of the organism (often 1.1). For example, the volume of a spheroid is $V=L*W^2*\pi/6$. V is converted to WW by assuming a density of 1.1. DW can be assumed to be 25% of wet weight (WW). After (Reiss and Schmid-Araya 2010).	where *V* = volume in mm^3 (i.e. microlitres); L = length in mm; W = width in mm; WW = wet weight in µg (as 1 µg = 1 mm^3); DW = dry weight in µg – converted to mg by multiplying with 1000	*Asellus aquaticus* specific equation from Flores et al. (2016*)* (mg, mm): $\log_{10}(M) = 2.652 * \log_{10}(L) - 1.841 = 6.4$ mg DW. If calculated as a spheroid then DW = 9 mg
Assemblage biomass (AB)	$AB = \sum M * N$	where *M* is individual body mass (e.g. dry weight, mg) and *N* is abundance (e.g. ind./m^2)	*AB* (mg) = 6.4mg *5 = 32 mg DW
Metabolic capacity (MC) of a consumer assemblage, based upon general allometric-body size scaling relationships	$MC = \sum$ (per capita $M^{3/4}$)	where *M* is individual body mass (e.g. dry weight, mg). The ¾ exponent used here describes a general relationship between basal metabolic rate and body size and has been applied to describe the allometric scaling of basal metabolic rate across wide range of organisms (see Brown et al., 2004; Peters, 1983).	*MC* (mg) = $\sum (6.4^{3/4})$ = 20 mg DW
Assemblage metabolism (I). I is similar to MC - but incorporates the effects of temperature. This equation yields predicted values for the expected rate at which resources should be consumed by each assemblage, based upon the sum of the metabolic capacities of all individual consumers in addition to the environmental temperature	$I = i_0 M^{¾} e^{-Ei/kT}$	where, i_0 is a normalisation constant that converts mass to energy and which is empirically derived, *M* = body mass, e = Euler's number, E_i is the activation energy of respiration (0.63 eV; after Gillooly *et al.,* 2001), *k* = the Boltzmann constant in eV per Kelvin and *T* = temperature in Kelvin.	This equation depends on results of a leaf decomposition experiment, i.e. depends on the empirical data and an empirically derived value for i_0; and hence we cannot give a worked example here - but see Perkins et al. 2010 and Figure 20.2 for example data. i_0 can be derived through calculating the anti-log of the intercept for the relationship between metabolic capacity and temperature corrected decomposition on a log-log plot.

Figures

Figure 20.1: a) Leaf decomposition in aquatic microcosms is strongly driven by the presence of leaf decomposers and abiotic conditions; b) the main drivers of leaf decomposition should be considered in leaf decomposition experiments; c) ecological theory provides some tools for anticipating the scale of leaf decomposition because it considers organism traits such as body size and temperature; d) laboratory experiments are designed to address particular questions, with particular leaf decomposers, and even complex set-ups can be run in logistically feasible ways.

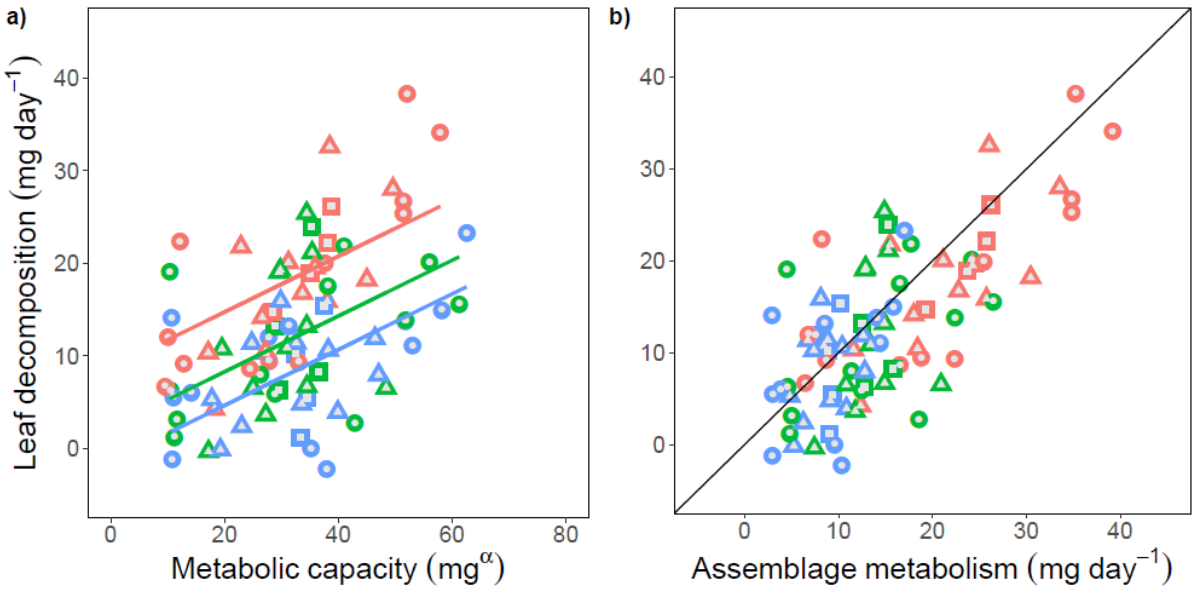

Figure 20.2: Metabolic capacity of invertebrate shredders and environmental temperature drive leaf decomposition in microcosm experiments. Data are redrawn from Perkins et al. (2010). Symbols denote species richness levels (circles = mono-cultures, triangles = di-cultures and squares = tri-cultures) and symbol colours represent temperature treatment (blue = 5°C, green = 10°C and red = 15°C). a) Leaf decomposition increases linearly with metabolic capacity with greater absolute rates (higher intercept values) with increasing temperature. b) When incorporating the effects of temperature through metabolic scaling principles (see equation in Table 20.2), observed leaf decomposition increases significantly with predicted rates (i.e. assemblage metabolism), whereby the slope of this relationship does not differ from the fitted 1:1 line (solid line).

Figure 20.3: Example for a statistical design of a leaf decomposition experiment (as used in Reiss et al. (2010). a) The 7 species used in the experiment are called 'types' and assigned a letter. The aim is to assemble them in mono, di- and tri-cultures to assess both the effects of species identity (i.e. the effect of 'type') and species richness. b) To reduce the number of microcosms, not all possible tri-cultures are run. Instead of 35 possible combinations, 7 are selected using the visualisation shown here. All types along a straight line, and along the circle are selected as tri-cultures and this means each pair of species occurs together exactly once. c) The latter results in a non-fully factorial design with 7 tri-cultures instead of 35. d) The questions will determine the statistical models that are fitted in the analysis of variance and the models can be visualised with a Hasse diagram. In these diagrams, there is one dot for each model and it is useful to show the dimension of each model – the number in the bracket, as well as its name. The diagram also contains edges linking some dots. The convention is that if model M1 contains model M2 then the dot for M1 is higher than the dot for M2 and there is a chain of generally downward edges linking the dot for M1 to the dot for M2. For example, 'Type richness' (3 parameters) and 'Type' (7 parameters) are not related models but they are nested in 'Assemblage identity' (35 parameters).

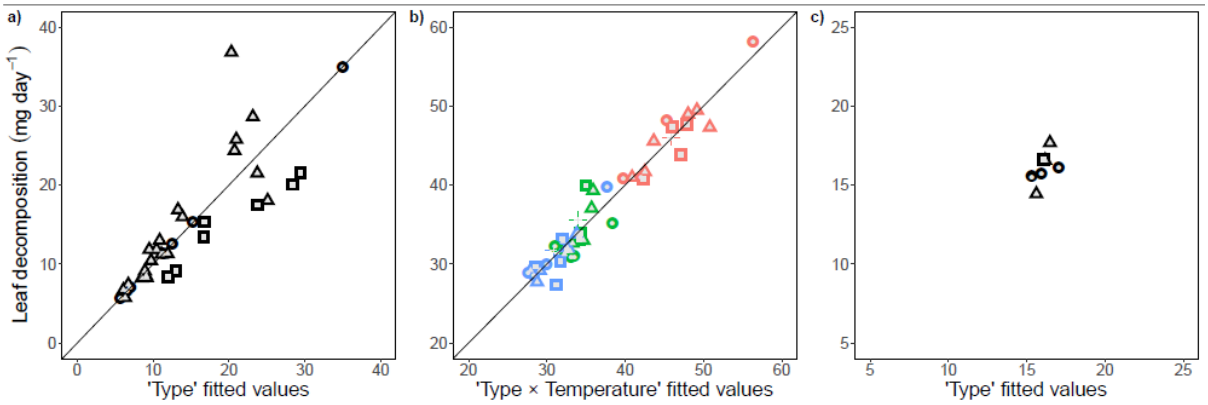

Figure 20.4: The performance of statistical model 'Type' in leaf decomposition experiments. This model assumes that each type has a unique effect, which provokes a characteristic response irrespective of whether the type is combined with other types or not. a) The type model describes the response extremely well in an experiment by Reiss et al. (2010). b) Perkins et al. (2015) ran a similar experiment at 3 different temperatures and fitting all combinations of type and temperature describes the data best. c) Data from Flores et al. (2016) showing that, when metabolic capacity is accounted for, the type model does not explain the data well because species effects that are based on body mass have been removed *a priori*. Each data point is a unique assemblage identity (averaged across replicates) where circles are mono-cultures, triangles are di-cultures, squares are tri-cultures and crosses are tetra-cultures. The fitted solid lines are 1:1 lines and are displayed in instances where the type model was significant in the original analyses.