Causal inference with observational data and unmeasured confounding variables

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**Code Repository:** <https://github.com/jebyrnes/ovb_yeah_you_know_me>

**App for one simulated dataset:** <https://shiny.umb.edu/shiny/users/jarrett.byrnes/shiny_ovb/>

**App for replicate simulations:** <https://shiny.umb.edu/shiny/users/jarrett.byrnes/ovb_sims/>

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**Abstract**

As ecology tackles progressively larger problems, we have begun to move beyond the scale at which we can conduct experiments to derive causal inferences. Experiments have long been seen as the gold standard for quantifying causal effects in ecological systems. In contrast, observational data, though available at larger scales, has primary been used to either explore ideas derived from experiments or patterns to inspire randomized controlled experiments, rather than for causal inference. This avoidance of using observational data for causal conclusions arises from the valid fear of results of unmeasured confounding variables in observational analyses. Unmeasured confounders that influence both the causal variable of interest and effect can bias conclusions - a problem known as Omitted Variable Bias. This phenomenon is what leads to the old saying, “Correlation is not causation.” Many other scientific disciplines, that cannot do experiments for reasons of ethics or feasibility, have developed robust approaches for causal inference from observational data. Here we show how Ecologists can harness these approaches, starting by using causal diagrams to identify potential known and unknown sources of confounding. We use a motivating example of assessing the effects of warming on intertidal snails to discuss how ecologists currently handle observational survey data and inference - often incorrectly. We present alternative sampling designs and the statistical designs that make use of them, discuss how they work using the language of causal path diagrams, demonstrate how easily they can be applied to common ecological datasets, and finally how well they are able to overcome problems of unmeasured confounding variables. We present these approaches to enable researchers to apply them to their own science as an important complement to experiments for generating meaningful insights into ecological systems.

Our goal is to enable researchers to advance the field of Ecology at scale using observational data.

**Introduction**

As Ecology advances to tackle problems at scales from the continental to global, we are putting our theories to empirical test like never before – working at larger scales in space and time and with unprecedented streams of data. To address fundamental questions in Ecology with these data, we desire to answer questions about causal relationships - either to test basic theory at scale or inform conservation and ecosystem management. Classically in Ecology, understanding causal relationships has been the domain of experiments. Experiments, however, have limitations for generalizing to larger scales and contexts beyond study conditions. As Ecology seeks to address theory and application at scale, we must rapidly move beyond a scale where ideal randomized experiments are possible (reviewed in Kimmel *et al.* 2021), and instead must be able to responsibly seize the opportunity of new large-scale sources of observational data.

Our ability to test hypotheses about causal relationships in observational data is limited by two fundamental challenges. First, nature is complex! When we use observational data to attempt to answer causal questions, we face numerous **confounding variables** – variables correlated with the cause and the outcome of interest – that can lead to incorrect estimates of causal effects (Fig. 1). Failing to control for these confounding variables leads to **bias** in our estimate of the relationship between a predictor and its response; the estimate will not be equal to its true value. A simple solution is to statistical control for confounding variables, but that requires knowing and measuring then. Even when we know confounders to account for, collecting all data needed to account for each and every one is likely impossible and measuring them with error can introduce other sources of bias. Second, as humans, we are limited by our ability to imagine how the different elements of complex ecological systems are related. Thinking through the entirety of the natural history of a system to design an analysis of observational data that will enable credible causal inferences is really hard. As a result, causal inference from observational data is often dismissed as impossible due to the potential for spurious correlations, prompting the common saying “correlation is not causation,” and the presentation of the correlation between number of pirates and global average temperature (REF?) as a cautionary tale of spurious correlations in observational data. At the core, inferring causation from correlations centers on dealing with the problem of unmeasured confounding variables – those that influence both a causal variable and the response of interest and can lead to spurious correlations or mask true causal relationships (Fig. 1).

A diagram of a driver causing a variable bias

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**Figure 1. Illustrating Omitted Variable Bias from Confounding Variables.** A response variable of interest (Y) is driven by both a measured variable (X) and an unmeasured variable (U), where (e) shows a random error term. In (A), X and U are uncorrelated, and thus the lack of inclusion of U in a statistical model increases the standard error of the estimate (decreases precision) of the effect of X on Y, but does not lead to bias. However, if U also drives X as in (B) or if U and X are driven by a common driver Z as in (C), then omitting U from a statistical model causes omitted variable bias in the estimate of the effect of X on Y. The direction of the bias in the estimator depends on the effect of U on Y, with the result that the causal effects are of X on Y are either over- or under-estimated, masking or mimicking a causal effect.

Excluding known but unmeasured, or unknown and unmeasured, confounding variables from a statistical analysis creates what is known as **omitted variable bias** (Rinella *et al.* 2020; Wooldridge 2015). Omitted variable bias (OVB) could be positive or negative and results in estimators yielding incorrect the magnitudes, and even signs, of estimates (i.e., biased estimators). Omitted confounding variables could occur because of missing measurements or due to failures of imagination – simply because we do not yet know confounding variables that are important. For example, one might measure plant communities to study competition, but not measure all the soil properties that drive all species, due to financial or time constraints. Similarly, working with long-term survey data or in human impacted systems, missing data on confounding variables is common, such as when using historical measures of fish abundance to study the impacts of changes in biogenic habitat availability, without measurements of fishing pressure during the same time-period. We have no way of knowing the direction or magnitude of the bias, because knowing all possible confounding variables and their relationships in a system is hard, if not impossible. Measuring, controlling for, and even knowing all potential confounding variables is nearly impossible in complex ecological systems (*reviewed in* Dee *et al.* 2023). In short, in observational data collection and analysis, we are always going to miss something, threatening the validity of our causal inferences.

*Do these challenges mean that we should not try to use observational data for causal inference?* We argue no. Rather than throwing up our hands, discounting and abandoning the use of observational data for causal inference, we suggest that ecologists consider adopting techniques from other disciplines that cannot do experiments – often for logistical or ethical reasons. For instance, it is not ethical to make a person smoke cigarettes daily to test the causal effect of smoking on dementia (Hernan & Robins 2023); one can only manipulate curricula so far in an effort to understand educational outcomes. Thus, disciplines such as psychology, economics, education, epidemiology, sociology, computer science, and more have been building tools to handle OVB in the causal analysis from observational data for decades (Angrist & Pischke 2008; Heckman 2000; Hernan & Robins 2023; Holland 1986; Imbens & Rubin 2015; Morgan & Winship 2015; Pearl 2009; Robins 1989; Rubin 1974, 2005). Indeed, these advances received the 2022 Nobel prize in Economics.

As ecologists, we have a decades-long tradition of considering experiments as a gold standard for causal inference (Benedetti-Cecchi & Cinelli 1997; Carpenter *et al.* 1985; Gotelli & Ellison 2012; Kimmel *et al.* 2021; Lubchenco 1980; Paine 1966; Power 1990; Reichman 1979; Silvertown *et al.* 2006; Underwood *et al.* 1997). However, experiments also rely on assumptions (Kimmel *et al.* 2021), which can be hard to meet in the field, induce artefacts, or rely on conditions that make them hard to generalize to natural ecosystems. Further, this reliance on the primacy of experiments has meant that the tools of other disciplines have been largely absent from the ecologist’s toolbox (*but see* Butsic *et al.* 2017; Grace 2021; Kendall 2015; Larsen *et al.* 2019; Rinella *et al.* 2020; Shipley 2016 for example). Recently, though, there is a growing interest and use of causal inference in ecology for observational data, including by drawing on a diverse suite of methods from other fields focused on casual inference (Arif & MacNeil 2022, 2023; Dee *et al.* 2023; Dudney *et al.* 2021; Grace & Irvine 2020; Larsen 2013; Larsen *et al.* 2019; MacDonald & Mordecai 2019; Simler-Williamson & Germino 2022). If we are, as a discipline, to move to more widespread use of observational data for causal inference, we need to carefully consider the problems of such approaches and the techniques we can use to mitigate them, and their assumptions.

Here, we aim to provide a guide to readily available ways to cope with Omitted Variable Bias (OVB) for Ecologists. We begin by briefly describing the status quo for how ecologists most often deal with omitted variable bias, including random effects, and their associated limitations. After, we review tools for identifying potential sources of omitted variable bias, building on foundation of using directed acyclic graphs that has become increasingly common in ecology (Arif & MacNeil 2023). We then outline sampling and statistical model designs for dealing with omitted variable bias. Most of these statistical model designs are underutilized, if not novel, for ecology. To illustrate problems with OVB and different ways to identify and address it, we present a motivating example that aims to quantify the causal effect of temperature on marine snail abundances. With this example, we demonstrate the conclusions that would be drawn from the typical approaches an ecologist might take with this data (e.g., random effects in a mixed model, *see* Bolker et al. 2009) – and why they fall short of dealing with OVB (i.e., have statistical bias) – compared to several other statistical model designs that can more adequately control for omitted variable. We then present results from simulation analyses showing that these designs – which have seen limited adoption in ecology – are more robust to OVB (i.e., they successfully eliminate more sources of bias). We provide guidance for choosing among these designs for different data contexts and questions, and hands-on tutorials with R code for prospective users. Our goal is to enable researchers to advance the field of Ecology at scale using observational data.

**How are ecologists coping with Omitted Variables Bias?**

Omitted variable bias is commonly dealt with in one of five ways in Ecology. The first is using randomized controlled experiments. In an ideal, randomized controlled experiment, the effect of confounding variables is eliminated when their assumptions are met (see Kimmel *et al.* 2021 on why this can be difficult in practice), because of the random assignment of treatments (e.g., Nitrogen addition) to units (plots), so that the treatment and control groups have the same level of any confounders on average. However, randomized controlled experiments, particularly at scale, are not always feasible and have limitations in terms of their ability to generalize beyond the experimental conditions.

Second, in observational studies, ecologists primarily attempt to deal with confounding variables by measuring the confounders and controlling for them in a multivariate statistical model. As described above, measuring all confounders, however, is often impossible -- particularly for retrospective analyses of existing data. Further, all potential confounders in the system might not be known. Third, ecologists sometimes make causal claims rooted in their knowledge of the natural history of a system. These claims can be problematic due to a lack of transparency and potential for incorrect statements of effect size; even the knowledge of the most accomplished naturalist can have gaps in their understanding of a system.

Third, ecologists often qualify their results verbally to avoid making causal claims – even when their research focus is causal understanding, rather than description (but see Laubach *et al.* 2021 on causal aims and claims). This practice muddies the waters and can create confusion over whether an author claiming an association or implying causation while allowing themselves plausible deniability. We feel that given our current need to understand causal relationships from large-scale observational data sets, these solutions are not adequate, and can even lead to misleading inferences.

Finally, ecologists use random effects in mixed-effect model frameworks (bolker et al). HOW MUCH TO SAY HERE? NEED TO INTRODUCE HERE

Ecologists have an opportunity and, nay, obligation, to leverage (or at least consider) the solutions to Omitted Variable Bias in causal data analysis that other disciplines have been building for decades. This paper provides an entry point into several approaches and complements recent reviews of what are commonly referred to **quasi-experimental methods** (e.g., Antonakis *et al.* 2010; Arif & MacNeil 2022; Bell *et al.* 2018; Bellemare *et al.* 2020; Butsic *et al.* 2017; the appendices of Dee *et al.* 2023; Ferraro & Miranda 2017; Grace & Irvine 2020; Kendall 2015) by expanding on panel designs for accounting for OVB.

**​​Using causal graphs to clarify causal assumptions and ferret out Omitted Variables Bias**

Causal diagrams -- or graphs -- (as part of. Structural Causal Models from Grace & Irvine 2020; see Arif & MacNeil 2023 for in depth introductions for Ecologists) are one of the first tools for identifying and addressing omitted variable bias (Arif & MacNeil 2023; Pearl 1995; Pearl *et al.* 2016). Causal graphs, or Directed Acyclic Graphs (DAGs), visualize our understanding of causal relationships and confounding variables within a system. In doing so, DAGs transparently clarify many assumptions on which one relies for making causal claims about relationships inferred from observed data, and potential sources of bias from confounding variables. Critically, a causal graph needs to include both measured and *unmeasured* confounding variables (and thus differs from a structural equation model, *ref)*. Finally, causal graphs can also show what variables you should *not* include in an analyses, such as those that cause collider bias – i.e., evaluating a relationship between two variables, but conditioning on something they both cause, such as looking for a relationship between disturbance intensity and herbivory intensity and conditioning on plant abundance, when the latter is caused by the two former (for an excellent discussion of this topic beyond the scope of this manuscript, see McElreath 2020 Chapter 6; Laubach *et al.* 2021; Griffith *et al.* 2020). Thus, we suggest drawing a DAGs before conducting an analysis from which one wants to make any causal conclusions.

If possible, we recommend making a DAG *before* data collection to inform which covariates might be confounding and should be measured if possible. However, due to feasibility constraints or if analyzing pre-existing data, measuring all potential confounders might not be possible. Further, the data could have been collected for another purpose or question, so a set of confounders were deemed unimportant.

**Box 1: A Brief Overview of the Elements of Directed Acyclic Graphs for Causal Analysis**

We briefly review the uses and the elements of causal diagrams (e.g., see Fig. 1), called Directed Acyclic Graphs (DAGs) (Arif & MacNeil 2023; Grace & Irvine 2020; Laubach *et al.* 2021; Pearl 1995). For the variables and implied causal relationships (as paths), we adopt symbology to differentiate between observed and unobserved variables to reveal where confounding variables might lurk. First, observed variables, which can be or have been measured, are represented as terms within boxes, as for *X* and *Y* in Figure 1. Second, our DAG in Figure 1 shows *unobserved* (i.e., unmeasured) variables contained in ellipses, such as the variable *U.* The error term is shown as *e*. Unknown confounding variables will be included in this error term (systematic error), along with random sources of error. Variables are connected by paths, i.e., arrows. The direction of these arrows represents a direct causal connection going in the direction the arrow is pointed. These arrows, unlike in a SEM or path analysis, are non-parametric. If the value of a causal variable of interest changes (i.e., via manipulation or intervention), there will be a concomitant change in the response variable(s) it affects. If a response variable changes, say via direct manipulation, there will be no associated change in the causal variable of interest.

A common critique is that DAGs do not include feedbacks, to which we respond by asking the reader to think of their definition of causality. Here we adopt the Neyman-Rubin counterfactual causality framework (Holland 1986; Rubin 1974, 2005) where we recognize that cause temporarily precedes effect. Therefore, feedbacks can be handled by thinking about a system with a temporal lag (e.g., Larson *et al.* 2008). If an instantaneous feedback is truly present, or if a time-series of both the driver and response variable is not available, one will likely require other tools such as instrumental variables - something beyond the scope of this manuscript (see Imbens 2014 for a comprehensive review).

In this article, we emphasize how thinking in terms of DAGs helps to determine where confounding variables might cause problems with omitted variables bias and, in turn, helps identify solutions in terms of sampling and statistical designs. As applied researchers, we have found that DAGs paired with robust statistical approaches for causal inferences have often clarified our own thinking and communication about ecological systems.

After building a DAG, as described in Box 1, one can determine potential sources of omitted variable bias from variables influencing both the cause of interest and outcome that have not been observed in the system (e.g., confounding unobservable variables, *U* in Fig. 1B). Not controlling for this confounding variable opens a “back-door” for information to flow between your causal variable of interest and its response variable via an unassessed pathway (Pearl 2009). In the case of Figure 1B, *U* would be folded into a statistical model’s error term, along with random sources of error. The model’s error term and causal variable of interest would be correlated, leading to endogeniety - a violation of a core assumption of Gauss-Markov (Abdallah *et al.* 2015; Antonakis *et al.* 2010).

What is endogeneity and why is it problem? Consider an example of evaluating the relationship between nitrogen availability and plant biomass across a series of fields. If nitrogen availability depends on field soil characteristics, and field soil characteristics also drive plant biomass, then 1) the effects of soil characteristics would be included in the error term so that 2) nitrogen is no longer an **exogenous** (external to the system of interactions) variable. Nitrogen is **endogenous**, meaning that it’s correlated with the error term (i.e., soil effects), and we are no longer only estimating the effect of nitrogen holding the effect of soil characteristics constant. Therefore, the estimate of the nitrogen effect will be wrong – different from the true effect in magnitude or even sign. Note, as discussed below, making field a random effect does not resolve this problem. With random effects, we estimate the variance parameter of fields, not their means; when we estimate the nitrogen effect, the effect of soils differing by fields is not accounted for. Any time a predictor is correlated with random effects – a statistical model will have an endogeneity problem. By drawing a DAG, we can see where those endogeneity problems are likely to occur.

One way to eliminate confounding from a variable (i.e., *U ),*  in the absence of an experiment, is to include the confounding variable in a statistical model to blocks all paths between *X* and *Y* via *U*, i.e.satisfying the **back-door criterion** (Pearl 1995, Fig. 2A). Controlling directly for a confounding variable “shuts the backdoor” – so that the causal variable and the error term are conditional independent (i.e. in expectation, the error term and causal variable are uncorrelated and no longer endogeneous). Depending on the causal relationships assumed for a system and data, there could be many confounding variables to measure and that need to be control for. Controlling for these measured confounders as covariates in a statistical model will “shut the back door” and enable causal identification through conditional independence (see Fig 2. and caption for several examples). Without including such confounding variables in a statistical model, this omitted variable will cause Omitted Variable Bias.

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**Figure 2.** **Examples of statistical control for confounding variables informed by causal graphs.** By including shaded observed variables, either U or W, in a statistical analysis of the effects of X on Y, omitted variable bias is controlled for the results have a causal interpretation. The relationship between the control variable and Y might (A and B) or might (C and D) not have a causal interpretation, depending on the structure of the system. Note, in (D), Q would have also served as an adequate control instead of W. R would have been a bad control.

DAGs can help identify how and when to control for confounding. With a diagram in hand, it can either be visually obvious or one can utilize a variety of software for analyzing DAGs (e.g., Textor *et al.* 2016) to find “open back-doors” (lack of conditional independence) that need to be controlled for to eliminate omitted variable bias. Software can also help us identify variables we should not control for – e.g., colliders. . Perhaps, most importantly, one can justify their choice of control variables with a DAG, making their assumptions about how a system works before an analysis transparent to readers of their work in the literature.

A causal diagram is, therefore, the first step on the way for identifying potential omitted variable bias. On their own, however, they do not in and of themselves provide a means for statistically controlling for OVB, particularly if we have not measured the confounding variable. Nor does a causal diagram help us in the face of *unknown* confounding variables that we have failed to imagine as part of our system. To address both issues, we must consider the designs of our sampling used to generate observational data and of the statistical models used with the data to produce causally identified estimates.

**A Problem of Omitted Snails**

To illustrate these empirical challenges and suite of potential solutions, we consider a marine benthic ecosystem, modeled after the Gulf of Maine, USA, where a researcher aims to study the causal effect of temperature on snail abundance. They hypothesize that temperature influences snail metabolic and mortality rates and wish to estimate its effect on snail population abundance. Snail population abundance is also driven by recruitment, in part influenced by regional oceanography (i.e., the flow of major currents and parcels of water that differ in a myriad of properties) that drives both water temperature and recruitment patterns (REF?). Let us assume that the researcher measured snail abundance and temperature at several sites, but not recruitment or any measurement of oceanography. Thus, recruitment and oceanography are unmeasured, or so-called “unobserved” confounding variables. Estimates produced from an analysis of just the temperature-snail relationship will almost certainly be or different from the true value. Even if the researcher had measured recruitment, though, what if there are other lurking confounding variables? Even if oceanography or recruitment were accounted for, omitted variable bias remains a real possibility – and the estimation of the effect of temperature on snails will be incorrect. Fortunately, our researcher drew out a causal diagram of the system (Fig. 3) and recognized that temperature at the scale of measurement was also influenced by local variation (e.g., small-scale oceanographic features, weather, or other sources of local or microclimatic variability). With this causal diagram (directed acyclic graph, Box 1) in hand, they realized they could control for both observed and unobserved confounding variables with appropriate sampling and statistical model designs, reviewed next.

## **A black background with a white circle Description automatically generated with low confidence**

**Figure 3.** **A causal diagram describing the controls of snail abundance in an intertidal ecosystem.** Oceanography drives both temperature and recruitment, both of which drive snail abundance. Temperature, however, is also driven by local influences as well. This could be variability in plot-level temperature within a site – i.e., sources of variation in microclimate - or site-level temperature variability over space or time uncorrelated with local oceanography, recruitment, or other site- or plot-level confounders.

## **Sampling Designs that enable statistical methods to cope with omitted variable bias**

Multiple sampling designs for data collection enable the use of statistical model designs that can address omitted variable bias from confounding variables that vary across space, time, or both. A key feature in these sampling designs is the **nesting** of multiple observations within a cluster or group (e.g. site), such that the causal variable of interest varies across replicates while the confounder varies at the cluster level (Fig. 4). Clustered data is often also referred to as a hierarchical or nested sampling design. We use these terms interchangeably. Using our snail and temperature example, we outline different nested sampling designs and discuss how they generate different source of variation in space and time that enable the use of statistical model designs that deal with confounders.

Nested sampling designs can take several forms and generate difference types of variation to study. First, a sampling design could include multiple plots sampled within sites at a single point in time (Fig. 4A) – a **cross-sectional design**. When sites span environmental gradients with variation in a causal variable of interest, confounding variables also vary across these spatial gradients. In our example (Fig. 3), a spatial gradient in temperature across sites also reflects the spatial gradient in oceanography that affects both temperature and recruitment, thus confounding this causal relationship of interest between temperature and snails across sites. However, with data collected from a cross-sectional sampling design, we can use variation in plot temperature *within*-sites to isolate its effect on snails rather than the variation *between* sites, which contains sources of confounding variation.

Second, one could sample the same plots (or sites) repeatedly through time (Fig. 4B) in a **longitudinal** or **panel data design**. This data structure enables using approaches that can leverage variation *within-sites through time*. As such, longitudinal data enable many approaches to remove the effects of confounding variables that vary across sites in a flexible way. Developing an understanding of how these two types of data structures can be used in conjunction with statistical designs to remove variation from confounders is key to confronting OVB.

A diagram of a design

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**Figure 4.** **Visual examples of hierarchical study designs with plots nested within sites sampled at one point in time in A and through time in B**. This figure shows sites distributed along a coastline with a corresponding thermal gradient, with plots sampled within each site. These sampling designs therefore have variation across space, as in the cross-sectional sampling design in A, or in both space and time as in B. which shows longitudinal or panel data, where the same plots within sites are observed through time. The sampling deisgn in (A) can allow researchers to study temperature variation within sites as well as between sites; however, there are potential many more confounding differences between sites than within sites. The design in (B) enables a researcher to leverage variation in space and time, including by examining variation within sites through time.

## **Statistical Model Designs to Coping with Omitted Variables**

With data and a DAG in hand, there are well-established statistical designs for analyzing clustered data to handle omitted confounders for causal analysis. We emphasize the term *‘designs*’ over *‘methods’* because one could implement these statistical designs using a variety of estimation approaches (e.g., linear regression, Generalized Linear Models, as a part of Structural Equation Models, or with Bayesian techniques). These statistical designs have different costs and benefits, and they differ in their assumptions required for interpreting an estimate as a causal effect. Yet, most of the following designs -- with the exception of random effects models as shown below -- allows us to flexibly control for confounding variables that are both known and unknown (see Angrist & Pischke 2008; Dudney *et al.* 2021; Ferraro & Miranda 2017) – something many Ecologists worry about. Thus, we believe these statistical designs are a key advance worth considering for ecologists.

We illustrate the different designs using a common set of terms for causal variables of interest *(x*; e.g. local temperature), the outcome or response of interest (*y*; e.g. snail counts), and confounding variables (*w*; e.g. recruitment) in a regression, applied to our example of the snail system in Figure 3. Our example includes data from different sites (*i*) sampled either at multiple time points (*t*) in panel data design or in multiple plots (*j*) in the case of a cross-sectional data design as above. For the sake of simplicity, we assume a linear model form with normally distributed error (), although the framework applies for generalized linear models as well, such that

(1) .

Here, *yij*is the abundance of snails at site *i* in year or plot *j*, is the intercept, is the effect of temperature *xij* at site *i* in year or plot *j* on snails, is the effect of recruitment *wi* at site *i* on snail abundance, Our goal is to estimate (the effect of temperature on snail abundance) without bias. Due to shared oceanographic influences, *xij* and *wi* are correlated. If we had measured *wi*, then we could include it in our model, and by conditioning on observables with as the effect of *w* on *y*, produce a causally identified estimate of , assuming no other confounders. Without measuring and including the confounder, *w*, in the design above, and then fitting the equation of

(2),

our causal inference about would be incorrect. This is because would now be included in the error term, inducing a correlation between our error and causal variable of interest. This **endogeneity problem** violates the assumptions of the Gauss-Markov theorem and its extensions (Wooldridge 2015) and is what underlies the problem of omitted variable bias (see simulations below to see this bias in action).

## *What Ecologists Typically Do: Random or Mixed Effects Models*

Mixed effects models have been popular in ecology for the past two decades (for a useful review, see Bolker et al. 2009, Schielzeth and Nakagawa 2012, Harrison et al. 2018). Originally used to partition variation in heritability between different relatives (Fisher 1919), **random effects –** the effects of clusters in data assumed to come from a random distribution (but see Gelman & Hill 2006 on the linguistic ambiguities surrounding fixed and random effects) – quickly became a mainstay in the partitioning of variation in randomized experiments with subsamples taken within clusters (Cochran 1937; Eisenhart 1947). They have become a standard part of the toolbox for analyzing ecological experiments (Schielzeth & Nakagawa 2012) and are frequently used when analyzing observational data in ecology.

Random effects account for clustering in data via the error structure of the model (Bolker *et al.* 2009; Gelman & Hill 2006), rather than estimating cluster means as part of the data generating process of a model (i.e., via fixed effect for each cluster’s mean, using the terminology of the mixed models literature). Random effects have the added second benefit of efficiency, costing fewer degrees of freedom to estimate as we assume cluster means follow from a distribution (i.e., estimating a grand mean and variance), rather than directly estimating a separate coefficient for each cluster mean with no relationship to any other cluster mean. With this efficiency can come an improvement in the estimates of *precision* for coefficient estimates for our causal variable of interest (Gelman & Hill 2006) relative to fixed effects cluster means. This improvement in precision contrasts to how cluster robust standard errors – a technique also designed to handle clustering in data - alters the precision of coefficient estimates. Cluster robust standard errors make no assumptions about the distribution of cluster and their means, and they make fewer assumptions about the homogeneity of residuals between clusters (see Box 4 and Oshchepkov & Shirokanova 2022 for an excellent comparison between mixed models and cluster robust standard errors including when and where to use each and data requirements). Further, as random effects are assumed to be drawn from a common distribution, they have benefits for analyses of unbalanced samples as well as regularizing of cluster means (i.e., shrinkage, drawing them towards the grand mean, see Efron & Morris 1975).

For these reasons, Ecologists conducting a study akin to our snail-temperature example would likely gravitate towards a mixed model to account for variation across sites in snail abundances, using a mixed effects model design like:

(3)

What is new here relative to eqn. 2 is that is the site-specific deviation at site *i* from our intercept due to random variation which follows a normal distribution. As we will see, because this is a random effect, if site is correlated with temperature, we cannot resolve the problem of OVB with this model.

*What assumptions is a random effects design making when it comes to omitted variables bias?*

Why does the above model not control for omitted confounders via its site effect? Why do mixed effects designs produce incorrect results in the face of omitted confounders (i.e., a statistically biased estimate of the causal effect)? To understand this problem, it is key to remember, that when we model random effects, we are not modeling group means *per se*. Rather, we are modeling correlation in our error structure due to clustering in our data (Bolker *et al.* 2009; Schielzeth & Nakagawa 2012; Wooldridge 2010). The coefficient estimates of the causal variable of interest are unaffected by including or not including a random effect (we recommend you try this with any demo data set you have lying around). This difference – modeling error instead of modeling means *per se* – results in many of the above benefits, but also introduces one new assumption not often considered – a variation on the assumption of endogeneity we call the**Random Effects Assumption**. This assumption states that the random effects, which are left in the error term, do not correlate with any covariates in the regression (Antonakis *et al.* 2021; Wooldridge 2010). In a mixed model in equation 3, the random effects of “site” are part of the error term and assumed to be uncorrelated with temperature for the random effects estimator to be unbiased (Schielzeth & Nakagawa 2012; Wooldridge 2010). In equation 3, while site is incorporated into the effect of temperature on snails is not causally identified and this estimator is biased due to the violation of the Random Effects Assumption; in short, estimates of will be wrong.

The case we describe above will be common in many ecological analyses when a causal variable of interest varies at the site level in a way that is confounded with other drivers occurring at the site level, i.e. and are correlated. This correlation violates of the Random Effects Assumption such that a random effects estimator will be biased.

A picture containing circle, moon, astronomy

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**Figure 5.** **The system assumed to be underlying a mixed model (A) versus the true system in (B**). DESCRIBE KKEY DIFFERENCE TO NOTE BETWEEN A AND B. Note that a mixed model does not account for the correlation between site and temperature, nor does it separate out the non-site drivers of temperature. Instead, the effect of temperature on snails is confounded by any correlated site-level drivers that correlate with temperature at the site level.

We can see more clearly how a mixed model would violate the random effects assumption in Figure 5a. In essence, site effects here are site-level residuals drawn from a normal distribution. They represent all other abiotic and biotic forces happening at the site level, but they also are assumed to all be uncorrelated with temperature at the site level. However, given the information in Figure 3, we know that this is not accurate, so the key assumption for an unbiased estimator is violated. If we were to take a step back and think about our analysis goals and our causal understanding, again representing unmeasured quantities in ellipses, what we have is more like Figure 5b. Here, while a random site effect would be wonderful in terms of all the benefits discussed above, we would need to remove the effects of site-level confounders to use it – which is not done with the mixed model design above, as shown in Figure 5a. This example illustrates the difficulty in satisfying the Random Effects Assumption*.* More generally*,* we posit that satisfying this assumption is often quite difficult in Ecology – particular in observational data that spans environmental gradients – yet how badly this assumption is violated the is not well explored or acknowledged widely enough. We need a solution that does not produce biased results due to violating assumptions.

*Enter the Econometric Fixed Effects Design*

The Econometric Fixed Effects Design represents a familiar starting point for many ecologists who are used to using categorical variables in ANOVA and ANCOVA (e.g., Gotelli & Ellison 2012). Before getting into some admittedly confusing language, we note that, for Ecologists, what this design is just using a categorical variable for cluster. The approach is that simple. To get further into the weeds, here we use Fixed Effect in two senses of the phrase to describe this model. The first is the use of the term “fixed effect” is drawn from the econometrics literature, where it refers to attributes of a system (e.g., site, plot, or year) that vary by cluster (i.e., a within cluster intercept) that are encoded in models as dummy variables. In Ecology, this as a categorical predictor representing site, block, or other descriptor of how our data is clustered. In our snail example, would be a site-level time-invariant categorical variable acting as a stand-in for recruitment. We also use “fixed effect” in the language of the mixed model literature – i.e., that the cluster means are estimated as part of the data generating process of the model, not as part of the random error component. We acknowledge that there are many uses and definitions of “fixed effect,” leading to a wealth of confusion with different uses of the term across fields ( Gelman & Hill 2006). We hope to not add to the confusion but note that both uses of Fixed Effects here are valid for this statistical model design.

Recognizing that confounding variables vary at the cluster-level, and thus by removing the effects of clusters we remove the effects of our confounding variables, we have two options to control for confounding and OVB. First, we can use a bit of algebra known as the **within transformation** or **fixed effects estimator** (Bell *et al.* 2018; Wooldridge 2010)and has some similarities to within-subjects centering in Ecology (van de Pol & Wright 2009). We illustrate this by manipulating the following equation:

(4)

where is our casual variable of interest, and the error term is composed of idiosyncratic (random error), , and , which represent differences across sites *i* including unmeasured confounding variables. To remove the effect of site-level confounding drivers, we transform the data by subtracting this average value from both sides across all years. On the right-hand side we can expand this to subtract . This leads to a transformed model which can generate a causally identified estimate of .

(5)

Using simple algebra, we have removed the confounding influence of time invariant, confounding variables for each site, whether they were observed or not. To achieve the same effect as this group means transformation (see Fig. 6A), we could instead use a model design with a categorical or so-called dummy variables for each cluster (i.e., a 0/1 encoding for each cluster, known as an econometric fixed effect). We can represent this as a site effect in a causal diagram (Fig. 6B). This design will control for omitted variable bias and produce identical results to the preceding model for (Angrist & Pischke 2008; Wooldridge 2010). For clarity, we can write this model either incorporating the dummy 0/1 variables (*x2i*) and site effects () or with just the site effect alone – i.e., means model notation (Gelman & Hill 2006). Note that unlike random effects in a mixed model design, is not constrained to be drawn from any predefined distribution. We present this without using treatment contrasts (i.e., with as a reference level and as the deviation from the reference level) for clarity.

(6)

Returning to our snail example, with site as an econometric fixed effect as in equation 6, we can control for different sites having baseline differences in their levels of recruitment due to other omitted variables that are also correlated with temperature – whether those confounding variables were measured or not (see Fig. 6b). Thus, this design allows us to relax the strong assumption that all confounding variables are observed and measured to interpret as causal when other assumptions are met (see Discussion). For ecological examples using this design, see Dee et al 2017 (Proc B). Dudney et al (2021), Ratcliffe et al. (2023), and Dee et al. (2023). to account for serial correlation, heteroskedasticity, and clustering of the error We return to a discussion of standard errors used for inference in Box 4.

The fixed effect design has some drawbacks, despite its simplicity and its strength in controlling for both observed and unobserved confounding variables. First, while fixed effect estimators make much weaker assumptions about confounding variables, these estimators are inefficient compared to random effects. For each fixed effect (each site in our example), we estimate a separate coefficient and thus are estimating more parameters and eating up degrees of freedom. We therefore need a larger sample size to achieve the same level of precision for our estimate using fixed effects versus random effects, presenting a bias-variance trade-off (Bell et al. 2018). However, in the case of omitted variable bias with the goal of causal inference, this framework is still preferable over a mixed model. Fixed effects make far weaker assumptions about our ability to observe, measure, and control for confounding variables compared to random or mixed effects. Second, we lose information about between-site variation, including gradients between sites that may be of interest, because this variation is absorbed in the fixed effects. These gradients, while confounded with other variables, could be the focus of some research questions which cannot be easily addressed using fixed effect model designs.

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**Figure 6.** Directed Acyclic Graph representations of different statistical models handling omitted variables in the text.

## *Group Means for Efficiency, Inference, Fun, and Profit*

To study between-site variation and mitigate the loss of efficiency from the fixed effect design, we can instead use **correlated random effects designs (REF NEEDED)**. Correlated random effects leverage and control for group means of our causal variable of interestto control for confounding variables. For every cluster (e.g., each site, year, or region), researchers calculate a group mean of the causal variable of interest to include as a predictor. These group means control for the effects of confounders at the cluster level thus acting as a proxy for confounders. Using group means of our causal variable also enables us to estimate a coefficient for between-cluster effects in our causal variable of interest, although these coefficients contain a combination of causal and confounded effects.

In Econometrics, this model design is known as a **Mundlak Device** (Mundlak 1978) and has many extensions (e.g., Wooldridge 2021). For clarity, we term it a **Group Mean Covariate** design. For the group mean covariate model design, we use the following equation:

(7)

where accounts for the effect of cluster-level confounders and is a random effect of that cluster (i.e., site). We can see what this looks like as a DAG in Figure 6c. From this diagram, we see that the site mean temperature is controlled for in estimating the temperature effect. The mean temperature of a site is estimated while controlling for each measured temperature.

The site mean temperature coefficient, called a **contextual effect** (Antonakis *et al.* 2021) in the Group Mean Covariate design, shows how changing the mean temperature of a site – and all properties that correlate with site mean temperature – would affect snail abundance were the temperature within a plot to stay the same. For example, *if our plot was 10 degrees C, what would snail abundance be if said plot was in a site with an average temperature of 5 degrees C versus 20 degrees C*? If the contextual effect is 0, then we can conclude that a simple mixed model would have sufficed and that omitted variable bias was not a problem in this particular analysis (Antonakis *et al.* 2021).

Finally, we must account for correlation in the error term when estimating standard errors used for statistical tests. to account for clustering in our errors.

The above statistical model design will run into problems, however, if the correlation between our causal variable of interest and its cluster-level mean is too high. To solve this, we can use a design that transforms our causal variable to interest to remove this correlation. We accomplish this with **Group Mean Centering**, where we subtract the cluster-level mean from the causal variable of interest. After this transformation, we use variation … We then use this cluster-level anomaly as our predictor variable alongside a cluster level mean as follows:

(8).

The coefficient on the site mean of temperature, , is the between-site effect of a driver of interest and confounders, and the anomaly from the site mean coefficient, , is the within-site temperature effect. Thus, e,Here, the interpretation of is different than in the Group Mean Covariate design. for our snail example is now a **between estimator** of the combined effect of moving across gradients in temperature and correlated drivers between the sites. If = , omitted variables are not meaningfully influencing snail abundances; both the between and within site differences are due solely to temperature or multiple confounders have perfectly cancelled one another out.

The Group Mean Covariate, Group Mean Centered, and Fixed Effects designs all differ in structure but they will yield the same point estimates of under most conditions and balanced data, as they all rely on within-site variation in temperature (see simulations below and Wooldridge 2010). Thus, one might ask: *which statistical design should I use*? This decision depends on the structure and size of one’s data (e.g., how many coefficients do you have the power to estimate given your sample size) and the question of interest (e.g., are you interested in between-site differences?). For example, do you have many sites and are only interested in the causal effect of temperature? Fixed effects design. Do you want to know how plot-level snail abundance would change if the average site temperature changes, but plot temperature stays the same? Group Mean Covariate design. Do you want to understand the effects of temperature while examining the net effect of many variables shaping between-site gradients? Group Mean Centered design. Each design can further be extended to cases where the magnitude of the causal variable of interest’s effect is moderated by the level of confounding variables (i.e., an interaction or “heterogeneous” causal effect, e.g.Box 2).

## *What a Difference Differencing Makes*

Our examples thus far have focused on confounding variables that are unobserved and vary across space (i.e., between sites). We have not discussed omitted confounding variables that differ across time. In the case of omitted confounders varying solely across time and not space (e.g., sites vary randomly in recruitment across space, but year-to-year regional variation in recruitment is correlated with year-to-year regional variation in temperature), we can extend the frameworks presented above, using years as we did sites as clusters. If time-varying confounders are uniform across sites (i.e., are additive with spatial confounders), then we can use an econometric fixed effect of time and an econometric fixed effect of space (a two-way fixed effect or TWFE model design, Wooldridge 2021) or a site-average of predictors and a time-average of predictors (a Two-Way Mundlak model design; Wooldridge 2021).

If, however, temporal confounders differ by site, we need a more general solution. If omitted confounders vary spatiotemporally, we can extend our previous framework further using the same principles (see Box 3 and Dee *et al.* 2023). If, however, temporal confounders merely vary in strength from one site to the next, the **first and second difference** statistical model designs provide easy solutions. These statistical designs deal with both spatial confounders and site-varying temporal confounders. To illustrate, consider extending our example so that, in addition to site-level oceanographic recruitment effects, the abundance of snails is influenced by coastal development over time at each site (Fig. 7A). However, rates of development are not the same across all sites. As such, separating the effect of local coastal development from the effect of local temperature variability on snail abundance is difficult. We can see this in a small modification to the dynamics of our system from eq. 1:

(9).

In this scenario, there are both site-specific confounders, represented by and temporal confounders. For our temporal confounder, is a site-specific trend in snails over time (*j*). If there is also a trend in temperature over time (e.g., climate change), our estimation of in any model that did not include our temporal-confounder would suffer from Omitted Variable Bias. One solution to this scenario is to fit a model with an econometric fixed effect of site to account for spatial confounders and a site by time effect to account for the confounding vsriables that are site specific and vary through time at the site level (Box 3). This would lead to twice the number of parameters as the number of sites, however, and might not be a feasible given data and power constraints.

A picture containing black, circle, darkness, astronomical object

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**Figure 7. Directed Acyclic Graphs representing the way that different designs handle spatial and temporal omitted variables.** A shows…. B…. C…. I THINK THIS NEEDS TO EXPLAON THE DIFFERENCES HERE MORE EXPLICITLY TO IT CAN BE READ AS STANDALONE.

Fortunately, there is a simple solution similar to the fixed effects transformation: **temporal differencing**. For each time point in our data, if we subtract the previous time point, we produce a model evaluating the relationship between change in our response variable versus change in our causal variable of interest. Like the fixed effects transformation, the confounding effects of site-level omitted variables that do not have a temporal trend are eliminated. After the transformation, remains as a term to be estimated with an econometric fixed effect, and it will recover an estimate of the trend for each site. This approach has the added benefit of sweeping up other unknown site-level trends into our estimate of . Our first difference model design, represented as a path diagram in Figure 7B, translates to the following with means model notation:

(10)

Here estimates the effect of temperature as before with estimating the site-level trend of other drivers. Note that confounders at the site level, above, are removed algebraically in this design. If there is no temporal trend in temperature, and as such there is no correlation with other site-level trends, we *could* use random effects for the site term. We caution, however, that this adds back the random effects assumption which is unlikely to be met. Note that if the time between sampling events is unequal across sites, we can divide change by time between samples to model change per unit time. Finally, if we are uninterested in site-specific trends, we can calculate the second difference which eliminates the need to estimate . Note that in this second differences statistical model design model is estimating the relationship between acceleration in change in temperature and acceleration in change in snails.

Using either temporal differencing design has several advantages. We again remove the effect of omitted confounders at the site level. We also control for or remove the effects of temporal confounders at the cluster level that have similar trends to our causal variable of interest. Thus, our estimate of a temperature effect is again causally identified. As we are handling two potential forms of omitted variable bias, our analysis is robust to omitted variable bias from two sources of unknown confounders. However, like the econometric fixed effect design, differencing removes *between site* variation, including unobserved and confounding differences across sites. Similarly, it cannot be estimated if the causal variable is time invariant and yields imprecise estimates if it changes little over time. The other main drawback of the differencing approach is the reduced sample sizes; we lose observations from one or two time periods. This reduction in sample size reduces power and can lead to less precise standard errors, especially in the case of the second difference design. However, this reduction in sample size could be outweighed by more robust and flexible control over confounders, both measured or unmeasured. Finally, temporal differencing approaches that take a second difference can account for cases with both spatial and temporal omitted confounders: a situation all too common in many real ecological systems. We outline how other designs can also be extended to account for spatial, temporal, and spatiotemporal confounders in Box 3.

## **Box 2: A Difficult Slope: Omitted Variables that Cause Variation in the Magnitude of the Causal Effect**

Frequently, an omitted confounder does not merely contaminate our estimate of a causal effect but can also lead to model misspecification in the form of missed heterogeneity in the causal effect. This occurs when the causal effect of our variable of interest depends on the level of the confounder itself (i.e., it modifies the causal effect – an interaction effect). In our example, consider that thermal effects in our snail system might depend on levels of recruitment because dense aggregations of intertidal organisms are often better at retaining water and thus resisting desiccation or other forms of thermal stress (e.g., Silliman *et al.* 2011). This is problematic if we have not measured recruitment. In a naive mixed model, we might incorporate this heterogeneity as a random slope. As before, however, the random effects assumption is violated, so a random effects estimator will be biased. To deal with the problem of omitted variable bias in this case, we present two solutions. First, we can use a fixed effects design and include an interaction term between the site dummy variable and our causal variable of interest, allowing us to estimate site-specific temperature effects. Given that we now have site-level slopes, the number of parameters can blow up, leading to this approach being highly inefficient and not advisable for small sample sizes. Instead, we could use correlated random effects approaches with an interaction between the group mean and our causal variable of interest. For example, for a group mean covariate (i.e. Mundlak device) design, we would use the following equation:

This design allows us to examine how site-level confounders – known and unknown – can lead to variation in the effect of our causal variable of interest. It can also show that they have no effect if the estimand for is not different from 0. We could use a similar model for the group mean centered design if deemed appropriate. If we suspect that the magnitude of the temperature effect varied with other non-confounded covariates, we could instead use a random slope. In general, models with interactions representing moderators can provide powerful insights into both the effect of the causal driver of interest as well as how those effects vary.

## **Comparison of Approaches**

To demonstrate the utility the preceding solutions, and the consequences of not using them, we fit a variety of models to simulated data based on a longitudinal study of snail populations at multiple sites based on Figure 3. For a single simulation run, we created a system as follows:

* Oceanography is a variable with a mean of 0 and standard deviation of 1.
* Site mean recruitment is -2 multiplied by the oceanography variable and then rescaled to have a mean of 10 individuals per plot (e.g., so it does not go negative). It is the same in a site across all years.
* Site mean temperature is calculated as twice the oceanography variable and then rescaled to have a mean of 15C.
* Site temperature in year t is determined by site mean temperature and additional variation, with a mean of 0 and standard deviation of 1.

Snail abundance at site *i* in year *t* is then determined in a given year by the following equation (adapted from eqn. 1) corresponding to Fig. 3: Snailsit = Recruitmentit + Temperatureit + Other Driversit with the effect of other drivers varying with a mean of 0 and standard deviation of 1. We then simulate sampling 10 sites over 10 years. We analyzed each simulation run using all the statistical model designs described above, compared to naive models with no site effect. We also included group mean covariate and group mean centered models with and without a random effect to demonstrate what a random effect in these models is doing with respect to estimating the standard error and handling unbalanced data.

We provide results from 100 simulated data sets created as above. Interested users can access all of the code in Appendix A or at https://github.com/jebyrnes/ovb\_yeah\_you\_know\_me. Appendix B and Supplementary Data 1 walk through the analysis of a single data set. For a more interactive exploration of this and the full suite of simulated data and parameters, see the web applications written using R Shiny provided as Appendix C (for one simulated run alone) and Appendix D (with 100 or more replicate simulations exploring the distributions of parameters).

A graph of different types of temperature

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**Figure 8.** **Distribution of point estimates of temperature effects from different models across all 100 simulations**. The true effect size (= 1) is highlighted with a dotted line. The Naïve model in equation 1, Random Effects model in equation 3, the Fixed Effects models in equations 5 and 6, the Group Mean Covariate models in equation 7and the Group Mean Centered models to equation 8, and the First Differences model to equation 10. **SAY KEY RESULTS .**

Broadly, our simulations show that the point estimates from the random effects (RE) model - what ecologists typically use - is consistently biased in these simulations and well-below the estimates from both the other designs and the true effect size (Fig. 8, Table 1). Further, not only is the estimated coefficient of the RE model always biased compared to other estimators in our simulations, it is more often within 2SE of 0 than all other models. In most simulations, the 95% confidence intervals of the RE model do not contain the true value of the temperature effect (Table 1).

**Table 1.** **Summary simulation results.** Mean and SD of point estimates of temperature effects from different models in the first two columns. Fraction of simulated runs where the mean +/- 2 SE of the temperature effect either overlapped 0 (i.e., high likelihood of committing a type II error) or did not contain the true effect of temperature in the final columns. Models are as in Fig. 8.

| **Model Type** | **Mean Estimate** | **SD Estimate** | **Fraction Sims where 95% CI Contains 0** | **Fraction Sims where 95% CI does Not Contain 1** |
| --- | --- | --- | --- | --- |
| Naive | 0.231 | 0.165 | 0.56 | 0.99 |
| Random Effects (RE) | 0.640 | 0.232 | 0.08 | 0.54 |
| \*FE Using Mean Differencing | 0.985 | 0.215 | 0.00 | 0.05 |
| FE with Dummy Variables | 0.985 | 0.215 | 0.00 | 0.05 |
| Group Mean Covariate | 0.985 | 0.215 | 0.00 | 0.05 |
| Group Mean Centered | 0.985 | 0.215 | 0.00 | 0.05 |
| Group Mean Covariate, no RE | 0.985 | 0.215 | 0.01 | 0.04 |
| Group Mean Centered, no RE | 0.985 | 0.215 | 0.01 | 0.04 |
| First Differences | 0.971 | 0.259 | 0.01 | 0.12 |

*\*FE = econometric fixed effects*

Other the naïve and random effects model, the other designs show equivalent estimates with balanced data (in Table 1). The group mean centering and group mean covariate designs are equivalent to the fixed effects approach for balanced data. In Appendix A, we investigate how unbalanced data effects the results from the group mean centering and group mean covariate designs. While the perform slightly worse (estimates of XXXX with SE of xxxx versus .985 with SEs of 0.215 in Table 1), they are still much closer to the true value of 1 than the random effects and naïve models. A second point from these additional explorations has to do with estimation of the standard errors and, therefore, the resulting statistical inference, when the data is unbalanced. In line with the benefits of random effects in mixed models, a site-level random effect improves the precision of estimates??? for Group Mean Centered or Group Mean Covariate models when either the study design is unbalanced or there is site-level variation that is uncorrelated with temperature (Appendix A). If our simulation has no site-level variation other than temperature and our confounder, a random effect does not improve either models’ ability to estimate the effect of our causal variable of interest. This assumption is unrealistic for most real data sets, however. As such, we highlight the need for a site level random effect with either of these two designs. For estimating standard errors, in general, we urge researchers to incorporate random effects or clustered robust standard errors as needed to accommodate clustering in the error, per the study design, recognizing the tradeoffs of using both and appropriate context (*reviewed in* Oshchepkov & Shirokanova 2022).

## **Box 3: Reality Bites: Coping with spatiotemporal omitted confounders**

Spatiotemporal confounding variables – those that are site (or plot) specific and vary through time – pose challenges, and the solutions require more thoughtful study and statistical model design. To illustrate, we consider a scenario where recruitment, a confounding variable related to both snail abundance and temperature, is not static through time but instead varies by site and year (as in a realistic case). For example, sites that experience strong cold-water pulses in a year also experience unusually snail high recruitment in those same years due to oceanographic drivers. The sampling designs for coping with spatiotemporal omitted variables are based on the same principles as cross-sectional and longitudinal sampling, only now we combine the two to include plots within sites that are sampled through time.

With longitudinal data with multiple plots sampled within a site through time, we can flexibly control for spatiotemporal confounding at the site level by extending the two-way fixed effect designs discussed above. We can add a site-by-time fixed effect, , to our model, in addition to a fixed effect of plot, , where *k* is a fixed plot within site resampled over time (see below for a discussion of fixed versus re-randomized plots). This produces the following means model:

From this equation, we can see that captures time invariant plot-level confounding effects while captures the effects of spatiotemporal omitted variables at the site by time level. Note, there could be additional spatial or temporal only confounders. This design sweeps their effects onto the spatiotemporal term.

In small datasets, the above model design can consume degrees of freedom rapidly. In datasets with insufficient power, we can instead use the correlated random effects (e.g., a variation on the Two-way Mundlak model design *sensu* Wooldridge 2021) which are more efficient. Correlated random effect use site-year means () and plot means () for the entire survey to control for spatiotemporal and plot confounding respectively:

Here the and terms are random effects for plot and unique site-time combinations respectively.

When sampling to handle spatiotemporal confounders, should plots within sites over time be permanent or randomly placed each year? The above models assume permanent plots, so we can eliminate confounding variables at the plot-level that is time invariant over the study period. For this reason, permanent plots help us cope with within-site OVB issues and have higher power to detect change over time (Urquhart & Kincaid 1999). Logistically, however, permanent plots within sites might not be possible. As such, the above models can be modified to drop plot effects; however, they would then assume that there are no confounding differences across plots and could have lower power to detect effects of drivers. We emphasize that the choice of fixed or random plot placement with these designs is a balancing act, however, as fixed plots can lead to a lower sample size due to logistical considerations in many environments, and direct readers to other explorations of this topic (e.g. Gomes 2022).

Finally, without variation within sites as well as through time – e.g., plots within sites resampled over years – we cannot include a site by year effect as in the above models. We can attempt to use site-level time trends (e.g., as linear or polynomial trends) or trends generated from Generalized Additive Models (Wood 2017) to approximate site-by-time effects (for a polynomial example to deal with confounding variables that are site specific but vary through time non-linearly, see Dee *et al.* 2016a), but, this requires knowledge of how the confounder varies at sites over time and extensive testing for robustness to these assumptions. In the many cases this is not possible or inadvisable given the likelihood of creating incorrect causal inference. In those cases, without multiple plots per site over time, “nothing to be done” (Beckett 1954).

In general, we urge caution when dealing with spatiotemporal omitted variables, and careful use of causal diagrams to ensure that we are controlling for a confounder at the relevant spatiotemporal scale. This topic is one that that deserves far more exploration in Ecology. More from other disciplines on this tricky class of problem and approaches can be found from literature outside of the scope of this paper (Athey & Imbens 2017; Ferraro & Hanauer 2014; Oster 2019).

**Discussion**

We hope that our introduction to statistical and sampling designs to address the problem of omitted variable bias and causal inference from observational data has shown that, through thinking carefully about biological systems, we can draw on a solid set of existing methods to solve this problem. Causal inference from observational data is possible. At the core of these techniques is building an *a priori* causal model of how a system works, and then using that model to guide your choice of sampling designs and statistical methods that you will use to answer your causal questions. Further, these techniques for addressing omitted variable bias are well within the standard statistical abilities of most modern ecologists (see Appendix B for implementation). The results of using the correct models, as seen in at least our toy example, can be profound for our ability to understand biological systems. It is time to begin using these tools to address some of the most pressing questions in the study of nature.

The concepts presented here can be viewed as part of a generalizable approach to handling confounding variables using clustered data. While we have talked of sites and years, the same concepts apply to studies with cohort effects, individual effects, or other lower levels of clustering as well as to larger-scale studies with not just sites and years but regions and decades. The general suite of approaches remains the same, and potential confounding variables at these different scales can be identified in initial causal diagrams. Cross-sectional and longitudinal sampling designs are generalizable beyond just the simple case presented in our simulation example. For instance, one could adapt the above designs if temperature and recruitment varied temporally at a regional rather than site scale (e.g., sampling plots within a single site or sampling many – both over many years to leverage spatial variation in temperature) or for spatiotemporal designs (*see* Box 3). Combining these sampling designs with others, such as the classic stratified random sampling design (Foster *et al.* 2018; Grafström & Lundström 2013; Kermorvant *et al.* 2019; Robertson *et al.* 2013; Stevens & Olsen 2004), will allow for the analyses that can improve causal identification and also provide more precision in estimation of causal effects over multiple environmental gradients. How to design a study to fully account for confounders, however, will hinge on a causal structure of the system. t

**Box 4: Clustered Robust Standard Errors: An Underutilized Tool in Ecology**

While the focus of this paper is on bias in the estimation of coefficients, many of the issues discussed overlap with issues of non-independence and other violations of assumptions that could generate incorrect standard errors and tests of statistical inference. In light of that, we recommend the use of clustered robust standard errors for some of the models above. Clustered robust standard errors are not commonly used in Ecology (but see examples and code in Dee *et al.* 2016; Dee et al2023 and in Appendix XXXX) despite being a way to continue to use Ordinary Least Squares and then flexibly apply a post-hoc adjustment to accommodate clustered data, heteroskedasticity, correlation between time points, and other arbitrary correlation structures within the data (Abadie *et al.* 2017; Cameron & Miller 2015). While random effects, autocorrelation structures in statistical models, and more, can address some of the same issues, robust standard errors often provide a simpler solution allowing researchers to not have to make more assumptions about the structure of their data that they are not interested in. There are tradeoffs, however, and as multiple techniques cover similar ground, we recommend looking at comparisons of approaches (e.g., Oshchepkov & Shirokanova 2022). A full discussion or review of robust standard errors is beyond the scope of this paper, but we refer applied researchers to the documentation for the ‘*sandwich’* package in R and other comprehensive reviews (e.g., Cameron & Miller 2015).

The important thing is to be transparent in how we deal or do not deal with the problem of confounding variables. What are the assumptions you are making to interpret an effect as causal? If you are using mixed models, do you meet the random effects assumption and why or why not? Have you evaluated your residuals to determine if you need to implement robust standard errors? Why did you include some covariates and not others? Do you have a DAG or even a conceptual model of your system that might help a reader understand your thought process? Putting these types of decisions into your work in even a brief sentence – if not a figure or full breakdown in a manuscript supplement (e.g., see Dee *et al.* 2023) – will go far in terms of making your analyses more transparent. It will make your work easier to be built upon to advance science. Even with this transparency, we also must be humble. We must accept that our models and knowledge are imperfect. Someday, someone will come along with a different approach that will produce different conclusions and yield new insights. This progression is part of the scientific process.

The approaches herein are not a panacea. They require assumption for causal inferences, as does any approach, including experiments (Kimmel *et al.* 2021). Some assumptions are shared with experiments: i.e., SUTVA – or the stable unit treatment value assumption which has two parts: 1) no interference or “spillovers” across units and 2) no multiple versions of or “hidden variations” in the causal variable of interest (reviewed in Kimmel *et al.* 2021). Most of the statistical model designs presented here also include assumptions that expected effects are linear and additive (Imai & Kim 2021) and homogeneous across units and time periods. We have included some discussion of relaxing these assumptions via interactions (i.e., Box 2); however, there is a growing literature on estimating causal effects under more varied forms of heterogeneity (Callaway & Sant’Anna 2021; de Chaisemartin & D’Haultfœuille 2020; Goodman-Bacon 2021; Sun & Abraham 2021). Relaxing this assumption takes more thought and consideration of one’s question of interest and the system dynamics from DAGs.

Further, all the approaches presented here make the parallel trends assumption. The assumption of parallel trends is most easily understood considering a binary causal driver of interest (i.e., if the driver is present or absent). It implies that, without driver being present, the *difference* in outcomes between different clusters (e.g. sites) after conditioning on any covariates present would be constant through time. This assumption is more likely met with fewer time periods (e.g., two time periods spanning before and after an impact, as in a BACI design). The assumption can be tested in the pre-treatment period but is untestable after the treatment (for details, see Roth 2022). This assumption extends to continuous causal variables. There, we assume the response of interest across clusters would have followed parallel trajectories in the absence of a change in the causal variable and adjusting for other observed covariates. The parallel trends assumption has come under a great deal of scrutiny recently (reviewed in Roth *et al.* 2023), particularly when changes in the causal variable of interest happen at different points in time across units (called “staggered treatments,” see Baker *et al.* 2022; Marcus & Sant’Anna 2021) and in the face of heterogeneous effects of causal variables (for details, see e.g. Borusyak *et al.* 2023; de Chaisemartin & D’Haultfœuille 2020; Goodman-Bacon 2021; Sun & Abraham 2021). This is a rapidly evolving literature, with many proposed solutions (reviewed in Roth et al. 2023), including for heterogeneous effects, non-linear cases, and continuous causal variables (Callaway *et al.* 2021). Many of these solutions are already being implemented in standard software (see Roth *et al.* 2023). Further, we suggest using the approaches reviewed here in concert with sensitivity tests (Altonji *et al.* 2005; Oster 2019; Rosenbaum 2002) by implementing multiple designs that make different assumptions to probe robustness of results (see Dee *et al.* 2023 for an ecological example).

Finally, we emphasize that this paper provides an entry point into a broader, interdisciplinary literature on causal inference in observational data, longitudinal data analysis, and panel regression methods. Indeed, other methods, such as instrumental variables and regression discontinuity designs, can be used to eliminate omitted variable bias when their assumptions are met (see reviews and examples in Angrist *et al.* 1996; Arif & MacNeil 2022; Butsic *et al.* 2017; Dee *et al.* 2023; Grace 2021; Kendall 2015). Thoughtful uses of the front-door criterion – the use of mediators between a cause and effect that are unaffected by confounders to resolve a causal relationship – might also prove useful for ecology (Bellemare *et al.* 2020; Pearl *et al.* 2016), although, as of yet, there are none to our knowledge in the Ecological literature. We urge ecologists, long grounded in experiments, to open themselves to writings in Econometrics, Epidemiology, Computer Science, Public Health, and other disciplines with rigorous approaches to causal inference in observational data. Embracing this transdisciplinary approach will enable us to enhance our knowledge of the tremendous advances in causal inference and explore questions currently beyond our reach. As an incomplete set of starting points for further reading, we recommend Cunningham’s Causal Inference: The Mixtape (2021), McElreath’s chapters on causal diagrams in Statistical Rethinking (2020), Angrist and Pishke’s Mostly Harmless Econometrics (2008), Morgan and Winship’s Counterfactuals and Causal Inference (2015), Sloman’s Causal Models (2005), and Pearl’s Causal Inference in Statistics: A Primer (2016). We also suggest Ecologists interrogate the assumptions and interpretations of their experiments (Kimmel *et al.* 2021). Given how an experiment was designed and run, are its results causally valid with respect to the purported mechanism? It is high time to critically interrogate how to get the robust causal inferences needed to grapple with our rapidly changing world.

## **Conclusion**

“Correlation does not equal causation” rings in many of our heads from our Biostatistics 101 courses. One main reason behind this message is the specter of Omitted Variable Bias from unmeasured confounding variables. This fear has impeded the use of observational data for causal inference in Ecology for much of its recent history. We hope this review can lift some of that fear and, armed with the tools introduced here and knowledge of a literature beyond this piece, we can move forward as a discipline. With a massively growing volume of observational data, problems at continental to global scales demanding rapid answers, and now, new arrows in our Ecological data analysis quiver, we look forward to seeing the studies and insights from the next generation of Ecologists.

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**References**

Abadie, A., Athey, S., Imbens, G.W. & Wooldridge, J. (2017). *When Should You Adjust Standard Errors for Clustering?* (Working Paper No. 24003). Working Paper Series. National Bureau of Economic Research.

Abdallah, W., Goergen, M. & O’Sullivan, N. (2015). Endogeneity: How Failure to Correct for it can Cause Wrong Inferences and Some Remedies. *Br. J. Manag.*, 26, 791–804.

Altonji, J.G., Elder, T.E. & Taber, C.R. (2005). Selection on Observed and Unobserved Variables: Assessing the Effectiveness of Catholic Schools. *J. Polit. Econ.*, 113, 151–184.

Angrist, J.D., Imbens, G.W. & Rubin, D.B. (1996). Identification of Causal Effects Using Instrumental Variables. *J. Am. Stat. Assoc.*, 29.

Angrist, J.D. & Pischke, J.-S. (2008). Mostly harmless econometrics. In: *Mostly Harmless Econometrics*. Princeton university press.

Antonakis, J., Bastardoz, N. & Rönkkö, M. (2021). On Ignoring the Random Effects Assumption in Multilevel Models: Review, Critique, and Recommendations. *Organ. Res. Methods*, 24, 443–483.

Antonakis, J., Bendahan, S., Jacquart, P. & Lalive, R. (2010). On making causal claims: A review and recommendations. *Leadersh. Q.*, Leadership Quarterly Yearly Review, 21, 1086–1120.

Arif, S. & MacNeil, M.A. (2022). Utilizing causal diagrams across quasi-experimental approaches. *Ecosphere*, 13, e4009.

Arif, S. & MacNeil, M.A. (2023). Applying the structural causal model framework for observational causal inference in ecology. *Ecol. Monogr.*, 93, e1554.

Athey, S. & Imbens, G.W. (2017). The State of Applied Econometrics: Causality and Policy Evaluation. *J. Econ. Perspect.*, 31, 3–32.

Baker, A., Larcker, D.F. & Wang, C.C.Y. (2022). How Much Should We Trust Staggered Difference-In-Differences Estimates?

Beckett, S. (1954). *Waiting for Godot: tragicomedy in 2 acts*. Evergreen book. Grove Press, New York.

Bell, A., Fairbrother, M. & Jones, K. (2018). Fixed and random effects models: making an informed choice. *Qual. Quant.*, 55, 117.

Bellemare, M.F., Bloem, J.R. & Wexler, N. (2020). The Paper of How: Estimating Treatment Effects Using the Front-Door Criterion.

Benedetti-Cecchi, L. & Cinelli, F. (1997). Confounding in field experiments: direct and indirect effects of artifacts due to the manipulation of limpets and macroalgae. *J. Exp. Mar. Biol. Ecol.*, 209, 171–184.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., *et al.* (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.*, 24, 127–135.

Borusyak, K., Jaravel, X. & Spiess, J. (2023). Revisiting Event Study Designs: Robust and Efficient Estimation.

Butsic, V., Lewis, D.J., Radeloff, V.C., Baumann, M. & Kuemmerle, T. (2017). Quasi-experimental methods enable stronger inferences from observational data in ecology. *Basic Appl. Ecol.*, 19, 1–10.

Callaway, B., Goodman-Bacon, A. & Sant’Anna, P.H.C. (2021). Difference-in-Differences with a Continuous Treatment.

Callaway, B. & Sant’Anna, P.H.C. (2021). Difference-in-Differences with multiple time periods. *J. Econom.*, Themed Issue: Treatment Effect 1, 225, 200–230.

Cameron, A.C. & Miller, D.L. (2015). A Practitioner’s Guide to Cluster-Robust Inference. *J. Hum. Resour.*, 50, 317–372.

Carpenter, S.R., Kitchell, J.F. & Hodgson, J.R. (1985). Cascading Trophic Interactions and Lake Productivity. *BioScience*, 35, 634–639.

de Chaisemartin, C. & D’Haultfœuille, X. (2020). Two-Way Fixed Effects Estimators with Heterogeneous Treatment Effects. *Am. Econ. Rev.*, 110, 2964–2996.

Cochran, W.G. (1937). Problems arising in the analysis of a series of similar experiments. *Suppl. J. R. Stat. Soc.*, 4, 102–118.

Cunningham, S. (2021). Causal inference. In: *Causal Inference*. Yale University Press.

Dee, L.E., Ferraro, P.J., Severen, C.N., Kimmel, K.A., Borer, E.T., Byrnes, J.E.K., *et al.* (2023). Clarifying the effect of biodiversity on productivity in natural ecosystems with longitudinal data and methods for causal inference. *Nat. Commun.*, 14, 2607.

Dee, L.E., Miller, S.J., Peavey, L.E., Bradley, D., Gentry, R.R., Startz, R., *et al.* (2016). Functional diversity of catch mitigates negative effects of temperature variability on fisheries yields. *Proc. R. Soc. B Biol. Sci.*, 283, 20161435.

Dudney, J., Willing, C.E., Das, A.J., Latimer, A.M., Nesmith, J.C.B. & Battles, J.J. (2021). Nonlinear shifts in infectious rust disease due to climate change. *Nat. Commun.*, 12, 5102.

Efron, B. & Morris, C. (1975). Data Analysis Using Stein’s Estimator and its Generalizations. *J. Am. Stat. Assoc.*, 70, 311–319.

Eisenhart, C. (1947). The Assumptions Underlying the Analysis of Variance. *Biometrics*, 3, 1–21.

Ferraro, P.J. & Hanauer, M.M. (2014). Advances in Measuring the Environmental and Social Impacts of Environmental Programs. *Annu. Rev. Environ. Resour.*, 39, 495–517.

Ferraro, P.J. & Miranda, J.J. (2017). Panel Data Designs and Estimators as Substitutes for Randomized Controlled Trials in the Evaluation of Public Programs. *J. Assoc. Environ. Resour. Econ.*, 4, 281–317.

Fisher, R.A. (1919). XV.—The Correlation between Relatives on the Supposition of Mendelian Inheritance. *Earth Environ. Sci. Trans. R. Soc. Edinb.*, 52, 399–433.

Foster, S., Monk, J., Lawrence, E., Hayes, K., Hosack, G. & Przeslawski, R. (2018). Statistical considerations for monitoring and sampling.

Gelman, A. & Hill, J. (2006). *Data Analysis Using Regression and Multilevel/Hierarchical Models*. Cambridge University Press.

Gomes, D.G.E. (2022). Should I use fixed effects or random effects when I have fewer than five levels of a grouping factor in a mixed-effects model? *PeerJ*, 10, e12794.

Goodman-Bacon, A. (2021). Difference-in-differences with variation in treatment timing. *J. Econom.*, Themed Issue: Treatment Effect 1, 225, 254–277.

Gotelli, N.J. & Ellison, A.M. (2012). *A Primer of Ecological Statistics*. Second Edition. Oxford University Press, Oxford, New York.

Grace, J.B. (2021). Instrumental variable methods in structural equation models. *Methods Ecol. Evol.*, 12, 1148–1157.

Grace, J.B. & Irvine, K.M. (2020). Scientist’s guide to developing explanatory statistical models using causal analysis principles. *Ecology*, 101.

Grafström, A. & Lundström, N. (2013). Why Well Spread Probability Samples Are Balanced. *Open J. Stat.*, 3, 36–41.

Griffith, G.J., Morris, T.T., Tudball, M.J., Herbert, A., Mancano, G., Pike, L., *et al.* (2020). Collider bias undermines our understanding of COVID-19 disease risk and severity. *Nat. Commun.*, 11, 5749.

Harrison, X.A., Donaldson, L., Correa-Cano, M.E., Evans, J., Fisher, D.N., Goodwin, C.E.D., *et al.* (2018). A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ*, 6, e4794.

Heckman, J.J. (2000). Causal Parameters and Policy Analysis in Economics: A Twentieth Century Retrospective\*. *Q. J. Econ.*, 115, 45–97.

Hernan, M.A. & Robins, J.M. (2023). *Causal Inference: What If*. CRC Press, Boca Raton.

Holland, P.W. (1986). Statistics and Causal Inference. *J. Am. Stat. Assoc.*, 81, 945–960.

Imai, K. & Kim, I.S. (2021). On the Use of Two-Way Fixed Effects Regression Models for Causal Inference with Panel Data. *Polit. Anal.*, 29, 405–415.

Imbens, G.W. & Rubin, D.B. (2015). *Causal Inference for Statistics, Social, and Biomedical Sciences: An Introduction*. Cambridge University Press, Cambridge.

Kendall, B.E. (2015). *A statistical symphony: instrumental variables reveal causality and control measurement error*.

Kermorvant, C., D’Amico, F., Bru, N., Caill-Milly, N. & Robertson, B. (2019). Spatially balanced sampling designs for environmental surveys. *Environ. Monit. Assess.*, 191, 524.

Kimmel, K., Dee, L.E., Avolio, M.L. & Ferraro, P.J. (2021). Causal assumptions and causal inference in ecological experiments. *Trends Ecol. Evol.*, 36, 1141–1152.

Larsen, A.E. (2013). Agricultural landscape simplification does not consistently drive insecticide use. *Proc. Natl. Acad. Sci.*, 110, 15330–15335.

Larsen, A.E., Meng, K. & Kendall, B.E. (2019). Causal analysis in control–impact ecological studies with observational data. *Methods Ecol. Evol.*, 10, 924–934.

Larson, D., Grace, J. & Larson, J. (2008). Long-term dynamics of leafy spurge (*Euphorbia esula*) and its biocontrol agent, flea beetles in the genus Aphthona. *Biol. Control*, 47, 250–256.

Laubach, Z.M., Murray, E.J., Hoke, K.L., Safran, R.J. & Perng, W. (2021). A biologist’s guide to model selection and causal inference. *Proc. R. Soc. B Biol. Sci.*, 288, 20202815.

Lubchenco, J. (1980). Algal Zonation in the New England Rocky Intertidal Community: An Experimental Analysis. *Ecology*, 61, 333–344.

MacDonald, A.J. & Mordecai, E.A. (2019). Amazon deforestation drives malaria transmission, and malaria burden reduces forest clearing. *Proc. Natl. Acad. Sci.*, 116, 22212–22218.

Marcus, M. & Sant’Anna, P.H.C. (2021). The Role of Parallel Trends in Event Study Settings: An Application to Environmental Economics. *J. Assoc. Environ. Resour. Econ.*, 8, 235–275.

McElreath, R. (2020). *Statistical rethinking: A Bayesian course with examples in R and Stan*. Chapman and Hall/CRC.

Morgan, S.L. & Winship, C. (2015). *Counterfactuals and Causal Inference*. Cambridge University Press.

Mundlak, Y. (1978). On the Pooling of Time Series and Cross Section Data. *Econometrica*, 46, 69–85.

Oshchepkov, A. & Shirokanova, A. (2022). Bridging the gap between multilevel modeling and economic methods. *Soc. Sci. Res.*, 104, 102689.

Oster, E. (2019). Unobservable Selection and Coefficient Stability: Theory and Evidence. *J. Bus. Econ. Stat.*, 37, 187–204.

Paine, R.T. (1966). Food web compexity and species diversity. *Am. Nat.*, 100, 65–75.

Pearl, J. (1995). Causal Diagrams for Empirical Research. *Biometrika*, 82, 669–688.

Pearl, J. (2009). *Causality*. Cambridge university press.

Pearl, J., Glymour, M. & Jewell, N.P. (2016). *Causal inference in statistics: A primer*. John Wiley & Sons.

van de Pol, M. & Wright, J. (2009). A simple method for distinguishing within- versus between-subject effects using mixed models. *Anim. Behav.*, 77, 753–758.

Power, M.E. (1990). Effects of Fish in River Food Webs. *Science*, 250, 811–814.

Ratcliffe, H., Kendig, A., Vacek, S., Carlson, D., Ahlering, M. & Dee, L.E. (2023). Extreme precipitation promotes invasion in managed grasslands. *Ecology*, e4190.

Reichman, O.J. (1979). Desert Granivore Foraging and Its Impact on Seed Densities and Distributions. *Ecology*, 60, 1086–1092.

Rinella, M.J., Strong, D.J. & Vermeire, L.T. (2020). Omitted variable bias in studies of plant interactions. *Ecology*, 101, e03020.

Robertson, B.L., Brown, J.A., McDonald, T. & Jaksons, P. (2013). BAS: Balanced Acceptance Sampling of Natural Resources. *Biometrics*, 69, 776–784.

Robins, J. (1989). The control of confounding by intermediate variables. *Stat. Med.*, 8, 679–701.

Rosenbaum, P.R. (2002). *Observational Studies*. Springer Series in Statistics. Springer, New York, NY.

Roth, J. (2022). Pretest with Caution: Event-Study Estimates after Testing for Parallel Trends. *Am. Econ. Rev. Insights*, 4, 305–322.

Roth, J., Sant’Anna, P.H.C., Bilinski, A. & Poe, J. (2023). What’s trending in difference-in-differences? A synthesis of the recent econometrics literature. *J. Econom.*, 235, 2218–2244.

Rubin, D.B. (1974). Estimating causal effects of treatments in randomized and nonrandomized studies. *J. Educ. Psychol.*, 66, 688–701.

Rubin, D.B. (2005). Causal Inference Using Potential Outcomes. *J. Am. Stat. Assoc.*, 100, 322–331.

Schielzeth, H. & Nakagawa, S. (2012). Nested by design: model fitting and interpretation in a mixed model era. *Methods Ecol. Evol.*, 4, 14–24.

Shipley, B. (2016). *Cause and Correlation in Biology: A User’s Guide to Path Analysis, Structural Equations and Causal Inference with R*. 2nd edn. Cambridge University Press, Cambridge.

Silliman, B.R., Bertness, M.D., Altieri, A.H., Griffin, J.N., Bazterrica, M.C., Hidalgo, F.J., *et al.* (2011). Whole-community facilitation regulates biodiversity on Patagonian rocky shores. *PloS One*, 6, e24502.

Silvertown, J., Poulton, P., Johnston, E., Edwards, G., Heard, M. & Biss, P.M. (2006). The Park Grass Experiment 1856–2006: its contribution to ecology. *J. Ecol.*, 94, 801–814.

Simler-Williamson, A.B. & Germino, M.J. (2022). Statistical considerations of nonrandom treatment applications reveal region-wide benefits of widespread post-fire restoration action. *Nat. Commun.*, 13, 3472.

Sloman, S. (2005). *Causal models: How people think about the world and its alternatives*. Oxford University Press.

Stevens, D.L. & Olsen, A.R. (2004). Spatially Balanced Sampling of Natural Resources. *J. Am. Stat. Assoc.*, 99, 262–278.

Sun, L. & Abraham, S. (2021). Estimating dynamic treatment effects in event studies with heterogeneous treatment effects. *J. Econom.*, Themed Issue: Treatment Effect 1, 225, 175–199.

Textor, J., van der Zander, B., Gilthorpe, M.S., Liśkiewicz, M. & Ellison, G.T. (2016). Robust causal inference using directed acyclic graphs: the R package ‘dagitty.’ *Int. J. Epidemiol.*, 45, 1887–1894.

Underwood, A.J., Underwood, A.L., Underwood, A.J. & Wnderwood, A.J. (1997). *Experiments in ecology: their logical design and interpretation using analysis of variance*. Cambridge university press.

Urquhart, N.S. & Kincaid, T.M. (1999). Designs for Detecting Trend from Repeated Surveys of Ecological Resources. *J. Agric. Biol. Environ. Stat.*, 4, 404–414.

Wood, S.N. (2017). *Generalized Additive Models: An Introduction with R, Second Edition*. 2nd edn. Chapman and Hall/CRC, New York.

Wooldridge, J.M. (2010). *Econometric analysis of cross section and panel data*. MIT press.

Wooldridge, J.M. (2015). *Introductory econometrics: A modern approach*. Cengage learning.

Wooldridge, J.M. (2021). Two-Way Fixed Effects, the Two-Way Mundlak Regression, and Difference-in-Differences Estimators.