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Using metabarcoding to ask if easily collected soil and leaf-litter samples can be used as a general biodiversity indicator

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ABSTRACT

The targeted sequencing of taxonomically informative genetic markers, sometimes known as metabarcoding, allows eukaryote biodiversity to be measured rapidly, cheaply, comprehensively, repeatedly, and verifiably. Metabarcoding helps to remove the taxonomic impediment, which refers to the great logistical difficulties of describing and identifying species, and thus promises to improve our ability to detect and respond to changes in the natural environment. Now, sampling has become a rate-limiting step in biodiversity measurement, and in an effort to reduce turnaround time, we use arthropod samples from southern China and Vietnam to ask whether soil, leaf litter, and aboveground samples provide similar ecological information. A soil or leaf-litter sample can be collected in minutes, whereas an aboveground sample, such as from Malaise traps or canopy fogging, can require days to set up and run, during which time they are subject to theft, damage, and deliberate contamination. Here we show that while the taxonomic compositions of soil and leaf-litter samples are very different from aboveground samples, both types of samples provide similar ecological information, in terms of ranking sites by species richness and differentiating sites by beta diversity. In fact, leaf-litter samples appear to be as or more powerful than Malaise-trap and canopy-fogging samples at detecting habitat differences. We propose that metabarcoded leaf-litter and soil samples be widely tested as a candidate method for rapid environmental monitoring in terrestrial ecosystems.

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1. Introduction

The environmental monitoring and indicator literature collectively calls for the efficient measurement of total biodiversity (or

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a substantial non-biased sample) (Andelman and Fagan, 2000; Cushman et al., 2010; Dolman et al., 2012; Edwards et al., 2014; Knight et al., 2008, 2010; Lindenmayer and Likens, 2010; Newton, 2011; Nicholson et al., 2012; Possingham et al., 2012; Stuart et al., 2010), which seem to be contradictory goals, given the infamous "taxonomic impediment" (Ebach et al., 2011). The impediment refers to the great logistical difficulties of describing and identifying species.

However, metabarcoding technology (Baird and Hajibabaei, 2012; Bik et al., 2012; Ji et al., 2013; Taberlet et al., 2012) is a strong candidate for achieving both goals. Metabarcoding combines DNA taxonomy with high-throughput DNA sequencing to identify mass samples of eukarvotes. Amplicons of species-discriminating 'barcode' genes from soil, water, or collections of organisms reveal the presence and, more noisily, the frequencies of species of fungi, plants, and animals (Bienert et al., 2012; Bohmann et al., 2014;

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Calvignac-Spencer et al., 2013; Fonseca et al., 2010; Hajibabaei et al., 2012; Hiiesalu et al., 2012; Ovaskainen et al., 2010; Thomsen et al., 2012; Yoccoz et al., 2012; Yu et al., 2012) and can recover ecological information in the form of alpha- and beta-diversity estimates (Fonseca et al., 2010; Hiiesalu et al., 2012; Yoccoz et al., 2012; Yu et al., 2012). Importantly, such collections are auditable, because sites can be sampled by independent parties and analyzed by certified entities following a standard protocol. Metabarcode datasets are also taxonomically more comprehensive, many times guicker to produce, and less reliant on taxonomic expertise (Baird and Hajibabaei, 2012; Bik et al., 2012; Ji et al., 2013; Taberlet et al., 2012). Finally, Ji et al. (2013) have shown that metabarcode and standard (=morphologically identified species) biodiversity datasets result in very similar management decisions for monitoring, restoration ecology, and systematic conservation planning. In sum, metabarcoding promises reliable, verifiable, taxonomically comprehensive, and cost-effective biodiversity measurement.

Despite this promise, however, considerable work remains before metabarcoding can be considered for widespread adoption. In this paper, we focus on a seemingly simple but very useful question. Is it possible to substitute ground-level (soil or leaf litter) samples for aboveground samples when conducting biodiversity surveys (Ibáñez et al., 2012; Taberlet et al., 2012)?

An important advantage of a ground-level sample is that it can be cheaply collected in minutes (followed by processing in the lab), whereas an aboveground sample, such as from canopy fogging or various traps (e.g. Malaise, flight-intercept, pitfall, light, and baited) are more expensive and can require days and multiple personnel, and traps are subject to theft, damage, vandalism, and deliberate contamination. For instance, we have observed colleagues losing Malaise traps to elephants and to children, leading to unbalanced sampling effort. The need to retrieve aboveground traps incurs extra field expenses and logistical complications. As a result, if an environmental-certification organization were to judge, say, whether a set-aside area were truly maintaining biodiversity (following the potential example of Ewers et al. (2011) for oil-palm plantations), aboveground traps would be problematic, because such samples could be deliberately adulterated by local managers (Newton, 2011; Meijaard & Sheil, 2012). In short, one-shot, ground-level samples, coupled with metabarcoding to overcome the taxonomic impediment, could let us squeeze out costs and possibilities for fraud in our monitoring data, thus accelerating environmental measurement.

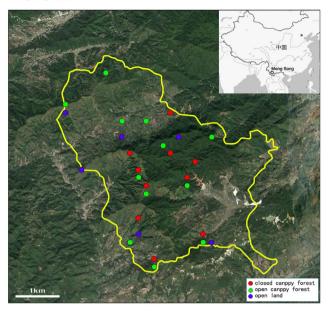
We have previously designed pipelines to metabarcode arthropod biodiversity from aboveground samples (Yu et al., 2012; Ji et al., 2013) and from soil and leaf litter (Yang et al., 2013). We now use these pipelines to compare soil and leaf-litter samples with Malaise-trap samples in southern China (Meng Song) and to compare leaf-litter samples with canopy-fogging and morphologically identified spider samples in central Vietnam (Vu Quang and Bach Ma). The samples were deliberately placed over a gradient of anthropogenic disturbance, and we ask if the different sample types all differentiate habitats in the same way.

2. Materials and methods

2.1. Meng Song, China

Meng Song is a village administrative unit in the Xishuangbanna prefecture of southern Yunnan, China (Fig. 1A, 21.5° N 100.5° E). The landscape includes part of the Bulong Nature Reserve, which is composed of seasonal montane rain forest and broadleaf evergreen forest (Zhu et al., 2005). The main cash crop is tea, which is grown as \sim 3 m tall understory trees in plots within thinned portions of the nature reserve (essentially, a kind of 'shade tea,' analogous to

A. Meng Song, China



B. Vu Quang (top) and Bach Ma (bottom), Vietnam

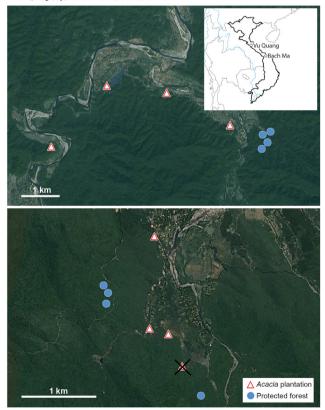


Fig. 1. Sampling maps. (A) Meng Song, China. Twenty-eight total samples are divided amongst 10 closed canopy forest sites, 12 open canopy forest sites, and 6 open land sites. (B) Bach Ma, Vietnam. (C) Vu Quang, Vietnam. Each Vietnam location was sampled in 8 sites: 4 *Acacia* plantations and 4 protected forests. Protected-forest sampling sites were clustered, due to limited accessibility. The sample marked with a black X indicates the sample that was omitted for contamination (Bach Ma – *Acacia*). (A) Meng Song, China.

shade coffee). Additionally, monoculture tea plantations have previously been cultivated on cleared land that consists of rows of tea shrubs in a matrix of grass, plus scattered individual trees and shrubs. In this landscape, 28 1-ha quadrats were established for a large biodiversity census project (Making Mekong Connected; Xu Download English Version:

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