Urban habitat restoration provides a human health benefit through microbiome rewilding: The Microbiome Rewilding Hypothesis

Running title: **Urban microbiome rewilding restores human health**

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ABSTRACT

Restoration aims to return ecosystem services, including the human health benefits of exposure to green space. The loss of such exposure with urbanization and industrialization has arguably contributed to an increase in human immune dysregulation. The Biodiversity and 'Old-friends' hypotheses have described the possible mechanisms of this relationship, and suggest that reduced exposure to diverse, beneficial microorganisms can result in negative health consequences. However, it is unclear whether restoration of biodiverse habitat can reverse this effect, and what role the environmental microbiome might have in such recovery. Here we propose the *Microbiome Rewilding Hypothesis*, which specifically outlines that restoring biodiverse habitats in urban green spaces can rewild the environmental microbiome to a state that enhances primary prevention of human disease. We support our hypothesis with examples from allied fields, including a case study of active restoration that reversed the degradation of the soil bacterial microbiome of a former pasture. This case study used high throughput amplicon sequencing of environmental DNA to assess the quality of a restoration intervention in restoring the soil bacterial microbiome. The method is rapid, scalable, and standardizable, and has great potential as a monitoring tool to assess functional outcomes of green space restoration. Evidence for the *Microbiome Rewilding Hypothesis* will help motivate health professionals, urban planners, and restoration practitioners to collaborate and achieve co-benefits. Co-benefits include improved human health outcomes and investment opportunities for biodiversity conservation and restoration.

Key words: ecosystem services; eDNA; immune dysregulation; metabarcoding; primary prevention; restoration genomics

Conceptual implications:

- We propose the *Microbiome Rewilding Hypothesis* – restoring biodiverse habitats in urban green spaces can rewild the environmental microbiome to a state that helps prevent human disease as an ecosystem service.
- Our hypothesis has potential to be a partial solution to the global megatrends of biodiversity loss and increasing immune dysregulation of urban populations.
- Aligning public health investments with restoration activities and biodiversity conservation will potentially lead to a frameshift in how these environmental activities are funded.

INTRODUCTION

The positive association between ‘green space’ and human health is well established (Speldewinde et al. 2015; Shanahan et al. 2016; Stein et al. 2016). However, the ecological and physiological mechanisms underpinning this relationship are not clear, and are likely to be multiple, interacting, and complex (Liddicoat et al. 2016; Nieuwenhuijsen et al. 2017). There is rapidly emerging evidence that the human microbiome is inextricably linked to health (Blaser & Falkow 2009; Cho & Blaser 2012; Weyrich et al. 2015). Indeed, the ‘Old-friends’ Hypothesis suggests that biodiversity provides an ecosystem service to humans as immunoregulatory health benefits accrue from exposure to the microbiome of biodiverse environments (Rook et al. 2003). The recently formed Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) assesses the status of biodiversity and the resulting ecosystem services that benefit society (https://www.ipbes.net/). Aligned with the goals of IPBES, conserved remnant environments and vegetation restored to maximize biodiversity could be valuable primary prevention tools – public health assets that prevent the onset, and reduce the incidence, of diseases. These biodiverse assets can potentially improve human health in urban landscapes, with the added benefit of conserving biodiversity.

In this paper, we review the available evidence for a *Microbiome Rewilding Hypothesis* (Box 1). Simply put, our hypothesis states that restoration of biodiverse habitat in urban green spaces can rewild the environmental microbiome to a state that benefits human health by primary prevention as an ecosystem service. We argue that testing this hypothesis is required to guide the provision of ecosystem services, including human health benefits, during ecological restoration. We propose that a new technique in restoration, high-throughput amplicon sequencing of

environmental DNA (eDNA metabarcoding), is an ideal tool to assess the environmental microbiome status of green spaces, and should be incorporated into restoration efforts to monitor the functional return of environmental microbiomes and their potential human health benefits.

The *Microbiome Rewilding Hypothesis* has knowledge gaps that require further testing (a subset is shown in Figure 1). For example, what are the associations between levels of urban biodiversity and healthy environmental microbiomes? Can restoration rewild urban environmental microbiomes? What portions of the environmental microbiome come from soil, air, leaf surfaces, and water? Lastly, what is the mode and degree of environmental microbiome transfer between the environment and humans? Should our hypothesis hold true, it will help lessen the human disease burden associated with urbanization (von Hertzen et al. 2011), while supporting restoration interventions (including additional financial investment) in places that typically have pronounced environmental footprints – such as big cities. Accurate and effective monitoring of the microbiome status of green spaces is essential to achieve this outcome.

BIODIVERSE ENVIRONMENTS PROVIDE HUMAN IMMUNE PROTECTION

The Biodiversity Hypothesis identifies a link between biodiversity loss, increased urbanization, and the increasing incidence of chronic inflammatory and psychiatric disorders in affluent urban populations removed from exposure to co-evolved ('old-friends') microbiota (Rook et al. 2003; von Hertzen et al. 2011; Kim et al. 2016). Since the 1950s, inflammatory diseases (e.g., allergic and autoimmune diseases) have become more prevalent in urban populations of affluent countries (Bach 2002; McDade et al. 2012). Recent medical research has revealed that many

of these inflammatory diseases are tightly linked to microbial imbalance (i.e., dysbiosis) of the human gut, suggesting that alterations in this microbiome are associated with their increased prevalence (Blaser & Falkow 2009; Blaser 2014).

Rural communities often have high immune protection against infections and diseases – the correlation of an environmental factor(s) with immune strength (Ege et al. 2011; Hanski et al. 2012). Evidence suggests that the immune systems of these individuals are primed by diverse antigens to adequately and appropriately respond to either pathogenic or harmless stimuli, thus training the immune system and decreasing the incidence of inflammatory diseases (Ege et al. 2011; Hanski et al. 2012). For example, high exposure to the microbe-rich rural environment of the Amish, a community practicing manual agriculture, confers strong immune protection, significantly more so than the rural Hutterites who practice mechanized agriculture (Stein et al. 2016). Rural children who live on farms, or within 5 km of forest or agriculture, have significantly lower prevalence of asthma and allergic sensitization to harmless environmental particles relative to urban children (Ege et al. 2011; Hanski et al. 2012; Ruokolainen et al. 2015). Moreover, the diversity of skin proteobacteria of rural children is higher than urban children, and correlates with increased expression of anti-inflammatory immune markers (Hanski et al. 2012; Ruokolainen et al. 2015). Additionally, animals raised in outdoor environments (with 'wilder' microbiomes) have stronger and more stable immune systems than genetically related populations raised indoors (Mulder et al. 2009; Flies et al. 2015).

THE *MICROBIOME REWILDING HYPOTHESIS*

We propose the *Microbiome Rewilding Hypothesis* – the restoration of biodiverse habitat in urban green spaces can rewild the environmental microbiome to a state

that benefits human health by primary prevention as an ecosystem service. Microbiomes are communities of microorganisms in a given environment (e.g., urban green spaces, humans) and rewilding, in ecological terms, refers to the allowance of natural processes to regain dominance over anthropogenic disturbances (Corlett 2016).

Microorganisms, both resident and colonizing, are immune system inducers and pacifiers, capable of both positive and negative immunomodulation that results in the adjustment of immune responses to normal levels in healthy mammals (Ichinohe et al. 2011; Fyhrquist et al. 2014). For example, microorganisms help guide immunoregulation by attenuating allergic airway responses by inducing regulatory T cells, and by stimulating memory-phenotype T cells for viral antigens that the host has not been exposed to (Karimi et al. 2009; Su et al. 2013). These health gains should be replicated by exposure to restored urban green spaces, given the effectiveness of restoration in recreating a biodiverse environmental microbiome (Gellie et al. 2017). Under our hypothesis, the relationship between plant communities and the environmental microbiome is core to restoring adequate human exposure to beneficial microbial communities.

The coevolutionary relationships between the soil microbiome and plants is well defined (Bonkowski et al. 2009; Van Nuland et al. 2016), and has been explored in the context of ecosystem restoration or recovery post-disturbance (Pansu et al. 2015; Cavagnaro et al. 2016; Hamonts et al. 2017). Correlated succession of soil microbial and plant communities has also been shown from both natural succession and restoration interventions (Figure 2; Banning et al. 2011; Rime et al. 2015; Gellie et al. 2017).

The ecological processes underpinning the correlated succession of plant and microbial communities are not fully resolved. However, there is clear evidence that plants shape the microbiome of their rhizosphere (Bonkowski et al. 2009). Different plant species often promote unique microbial communities when grown in the same soil type (Berendsen et al. 2012; Geremia et al. 2016). Additionally, a single plant species will promote the same soil bacterial community when grown in different soil types (Bulgarelli et al. 2012; Lundberg et al. 2012). Moreover, when symbiotic microbes are absent from degraded soils, plant community development can be hampered until an appropriate soil community is incorporated through inoculation (Wubs et al. 2016). Utilizing host phenotypes to artificially select an associated microbiome beneficial to host fitness has been proposed as a method to engineer microbiomes (Mueller & Sachs 2015). These examples provide evidence of the causative links between plant and soil communities and provide methods for beneficial manipulation of this relationship with great potential in urban rewilding.

Human immunoregulation evolved in our biodiverse ancestral environments, with both pathogens and commensal ‘old-friends’ priming and maintaining our innate immunity, adaptive immunity, and commensal microbiota (Rook et al. 2014). Continuous exposure to the natural environment results in appropriate succession of gut microflora and stable immunoregulatory development (Mulder et al. 2009; Flies et al. 2015). Therefore, removal of this exposure in urban societies likely contributes to immune dysregulation.

MONITORING MICROBIOMES IN URBAN GREEN SPACES

Responses in the soil microbiome from revegetation have traditionally been explored with broad-spectrum or indirect methods. For example, Li et al. (2015) used

183 phospholipid fatty acid profiles to show that native *Cupressus* trees restored soil
184 microbial communities at a faster rate than exotic *Eucalyptus* trees at a phosphate
185 mine restoration site in China. Additionally, Banning et al. (2011) used 16S rRNA
186 microarray analysis to show that restoration of a bauxite mine in a temperate climate
187 took soil communities approximately 10 years to restore after replanting the native
188 plant community. Unlike these previous studies, Gellie et al. (2017) recently used
189 eDNA metabarcoding over a 10-year restoration chronosequence of a former
190 pasture. The work of Gellie et al. (2017) explored the effectiveness of replanting the
191 native plant community in returning the soil microbiome to a more native state
192 (Figure 2). Metabarcoding amplifies and sequences a single gene region (amplicon)
193 from an environmental sample (e.g., soil, air, leaf surfaces, human swabs). PCR
194 primers have been developed to discriminate operational taxonomic units (OTUs)
195 within a target taxon (e.g., the 16S ribosomal RNA encoding locus in bacteria, Lane
196 1991). The approach can be used to describe microbial communities in OTUs and
197 higher taxonomic levels (e.g., phyla, classes, genera), thereby producing useful
198 information on the level and turnover of diversity in samples (Tedersoo et al. 2014;
199 Young et al. 2014).

200 Gellie et al. (2017) observed clear changes in the bacterial community after
201 just eight years of native plant restoration, whereby the bacterial communities in
202 younger sites were more similar to cleared sites and older sites were more similar to
203 remnant stands (Figure 2). The revegetation of the native plant community strongly
204 impacted on the belowground bacterial community, despite the revegetated sites
205 having a long and somewhat altered land use history (more than 100 years of
206 grazing). The changes detected in the microbiome were consistent with this restored
207 community being of greater potential for improving human health than unrestored

sites. Nieuwenhuijsen et al. (2017) called for standardized methods of green space quality assessments. Gellie et al. (2017) demonstrated that eDNA metabarcoding is a cost-effective, scalable, and standardizable ecological monitoring tool, which we recommend should be implemented in urban green spaces to explore microbiomes in these human-dominated environments.

eDNA metabarcoding holds great promise in microbial ecology, and its use for biodiversity assessment from environmental samples has increased markedly with far-reaching applications (Thomsen & Willerslev 2015). The principal reason for this rapid growth is that this approach gives a well-rounded description of the environmental microbiome, which is not easily assessed using microscopic or culture methods. Despite these benefits, very few studies have applied this approach to restoration or urban green spaces (Mhuireach et al. 2016; Gellie et al. 2017; Hamonts et al. 2017), perhaps due to its perceived high costs and technical challenges.

Three principal issues with eDNA metabarcoding are the difficulty in distinguishing lower taxonomic levels (e.g., species), accurately measuring biological abundance from amplicon read abundance, and sample contamination. Evolutionary divergence between species for a single amplicon (e.g., bacterial 16S rRNA) is not always sufficient for metabarcoding to accurately differentiate species, as compared with metagenomics (Moore et al. 2006; Wang et al. 2011; Oliveira et al. 2013; Kim et al. 2014; Zhang et al. 2015). However, the power to identify species increases when multiple amplicons are targeted (Wang et al. 2014; Beckers et al. 2016). As such, most metabarcoding studies limit species inferences and focus more on biodiversity classified at higher taxonomic levels.

Another issue is that OTUs don't necessarily translate to biological abundance (Smets et al. 2016). However, by setting stringent internal standards, confidence in abundance estimates can be increased (Smets et al. 2016). For example, the OTU read abundance used to calculate bacterial abundance based on community metrics, tend to scale data to relative abundance or employ diversity indices (Banning et al. 2011; Rime et al. 2015).

Moreover, metabarcoding approaches can be hampered by contamination of laboratory reagents, lab ware, sampling devices, and environmental exposure, as the amplification methods used prior to sequencing are sensitive (Salter et al. 2014; Llamas et al. 2017). An assessment of contamination is critical for obtaining clean signals between samples and sites. Despite these potential shortcomings, metabarcoding has proved effective in monitoring biodiversity changes in several systems, as outlined above. Its cost-effective, scalable, and standardizable nature makes it an ecological monitoring tool that has potential to be superior to traditional field-based monitoring (e.g., PLFA, culturing, microscopy), particularly for microbial diversity.

TESTING THE *MICROBIOME REWILDING HYPOTHESIS*

Gellie et al. (2017) demonstrated that restoration has the potential to rapidly (within 10 years) return a natural environmental microbiome, with potential for added health benefits to exposed populations. The human health potential of biodiverse plantings presents an imperative to rigorously test the *Microbiome Rewilding Hypothesis*. However, several knowledge gaps (Figure 1) need to be addressed before biodiverse plantings can become a global immune-health intervention.

It is still not known whether it is important for urban green spaces to reflect remnant vegetation – do manicured lawns, unkempt lots, managed parklands, restored native plant communities, and remnant plant communities have significantly different health-related microbiomes? Therefore, determining the level of macro-diversity required to engineer a healthy environmental microbiome will help guide the level of investment required for urban microbiome rewilding. Additionally, what is the contribution, and how does exchange take place, between microbiomes of different components of urban green space environments (e.g., soil vs. air vs. leaf surfaces)? Hence, quantifying the microbiomes of soil, air, and leaf surfaces with comparable methods will help to identify which aspects of green spaces are key for human interaction. Thus, what is the pathway and level of exposure required for significant interplay of microbes between urban green space environments and human environments to take place? Therefore, exposing human subjects to varying levels of macro-diversity will allow quantification of the environmental contribution to the human microbiome.

Urban green space access correlates with increased physical activity and lower occurrences of depression and high blood pressure (Shanahan et al. 2016; Liu et al. 2017). However, urban green space recreation potentially exposes users to harmful pollutants. For example, Ke et al. (2017) demonstrated that polycyclic aromatic hydrocarbons in urban park soils of Guangzhou, China, predominantly originated from vehicle and coal emissions, while 79% of parks studied had a high cancer risk to users. However, other urban environments were not measured for cancer risk comparison in this study. Indeed, pollution can change the environmental microbiome (Simonovicova et al. 2017), therefore pollution monitoring and amendment will be important in urban microbiome rewilding. Pollution and user

activities are potential confounding factors to microbial exposure for health outcomes, and should be controlled for in experiments testing the *Microbiome Rewilding Hypothesis*.

The trends and issues impacting human health in the Biodiversity Hypothesis are of global concern, and there is a need to research the *Microbiome Rewilding Hypothesis* in cities across the world as a means of providing solutions. Addressing the knowledge gaps outlined above will require a concerted research effort, and we are beginning some of this. For example, vegetation surveys that are synchronized with eDNA metabarcoding of urban green space types and human subjects is required. Extending this body of work across several cities, especially across a variety of stages of development, will be key to understanding the impact this pathway may have on affluent vs. underprivileged peoples. Testing the *Microbiome Rewilding Hypothesis* will guide restoration interventions that could potentially provide primary prevention for human health benefits, helping to generate additional financial investment for biodiversity conservation and restoration from the health sector.

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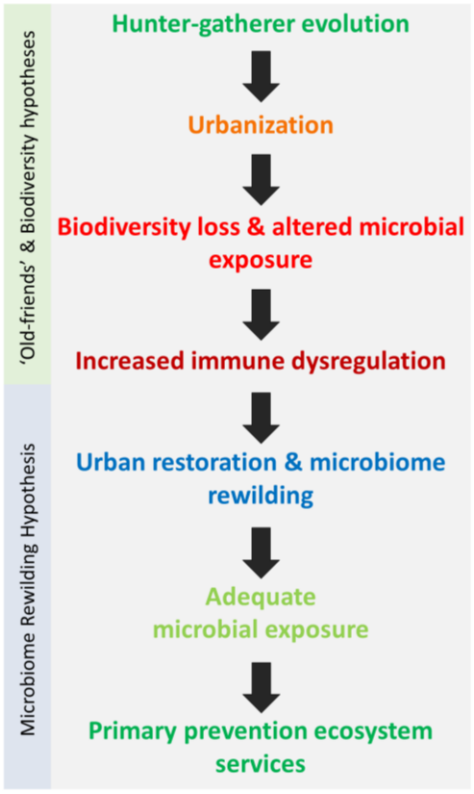
Box 1 The *Microbiome Rewilding Hypothesis*

The 'Old-Friends' and Biodiversity hypotheses propose that the increase in immune dysregulation in westernized urban populations is due to the disjunction between microbial exposures in urban vs. rural settings, which is being driven by the global megatrend of biodiversity loss. Numerous empirical studies support these hypotheses (see text).

We propose the *Microbiome Rewilding Hypothesis* – restoration of biodiverse habitat in urban green spaces can rewild the environmental microbiome to a state that benefits human health by primary prevention as an ecosystem service. This hypothesis proposes that ecological restoration of urban green spaces can potentially return the coevolved ecosystem service of immune protection provided by the microbiomes of natural environments, jointly decreasing the global megatrends of biodiversity loss and immune dysregulation.



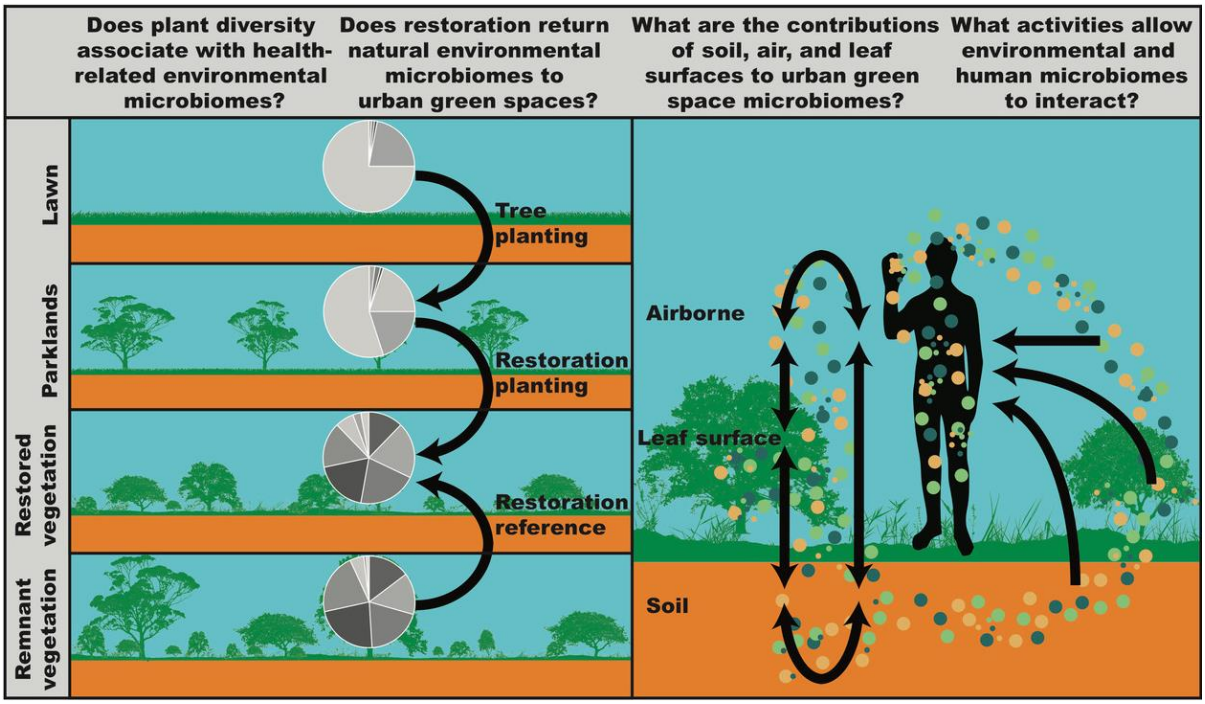
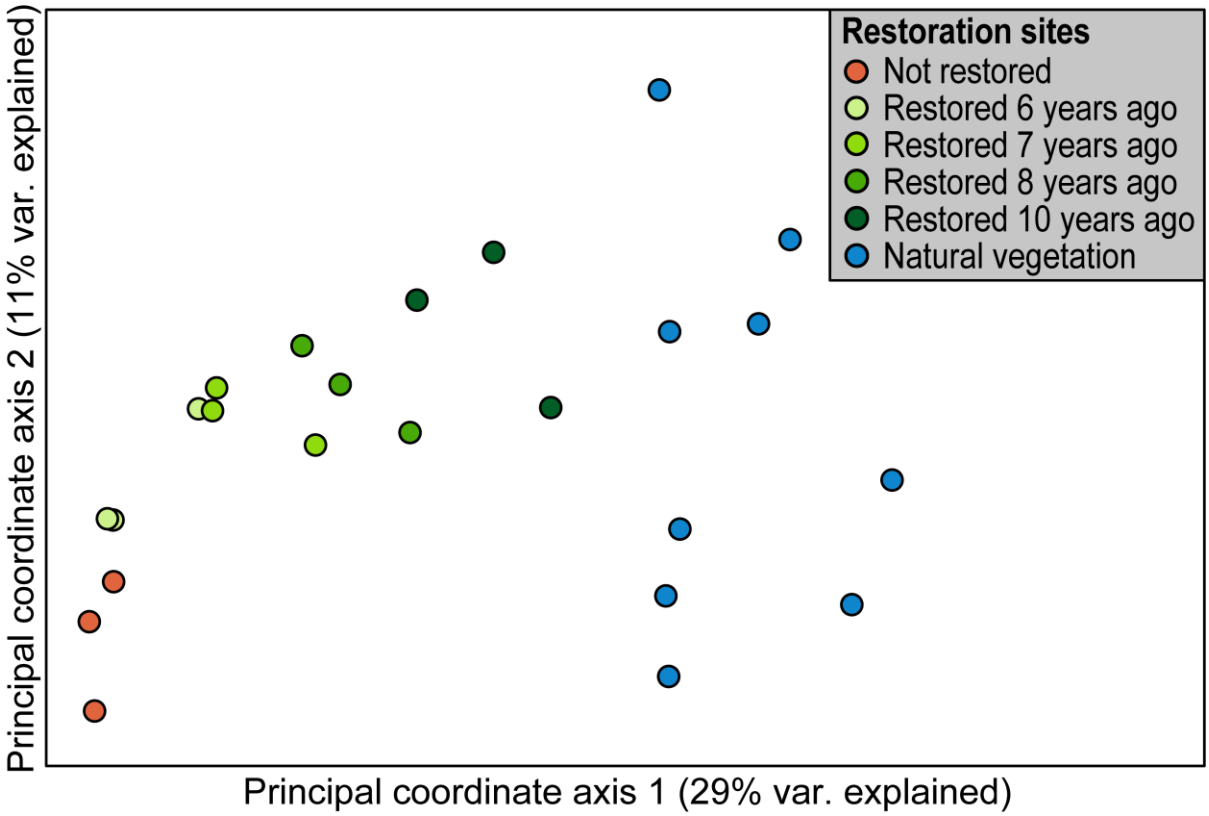


Figure 1 Some key knowledge gaps of the *Microbiome Rewilding Hypothesis*.



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474 **Figure 2** Rewilding the bacterial microbiome of a former pasture by replanting native
475 vegetation. The soil bacterial community in restored sites displayed a clear trend
476 away from cleared sites and towards closely resembling natural vegetation within 8
477 years of restoration. Figure from Gellie et al. (2017).