Rapid Ecological Restoration for Aggregate Sites: Replication of Mature Hardwood Forests

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1.0 EXECUTIVE SUMMARY

1.1 Aggregate producers require knowledge of, and access to, the best practices in ecosystem restoration if they are to excel on multiple fronts: as good neighbours and stewards, but also competitive in the market, able to reliably meet environmental commitments and thereby forge positive relationships with regulators. Best ecosystem management practices can ensure post-extraction rehabilitation goals are fully met, and increasingly, assist in the implementation of forest-creation (afforestation) or other activities outside of extraction areas which are aimed at offsetting industry impacts on climate, biodiversity, habitats and ecosystem services. Although afforestation initiatives are gaining huge momentum globally, ecologists are alarmed that in many cases, afforestation is being implemented in ways which can cause more harm than good. One major concern is that although much of the ecological value of existing forests stems from the powerful “co-benefits” produced – e.g. aside from carbon sequestration, woodlands provide habitat for rich biodiversity, support for pollinators, recycling of water and nutrients, resilience to climate fluctuations, and much more – these boons are not produced nearly so well in planted forests as they are in spontaneous tree stands developing by ecological succession over centuries. Understanding how to manage environments in ways that maximize production of co-benefits associated with mature spontaneous ecosystems is an urgent issue much broader than the aggregates industry.

1.2 Fortunately, TOARC, Walker Industries Inc., and other collaborators in Ontario’s aggregates industry have been in a unique position to support innovative research aimed at discovering practical techniques environmental managers may deploy to produce high-quality habitat and support biodiversity consistent with late-successional hardwood forests. We created the Rapid Ecological Restoration for Aggregate Sites (RERAS) study to test predictions generated by a “transplant surgery for ecosystems” paradigm. We hypothesized that valued properties of ecosystems which normally require long durations to develop may be stimulated to emerge from recently disturbed environments provided that the outcome of succession is treated more like a vital organ which can carefully transplanted than a machine to be built or repaired. Principles of successful organ transplantation in humans dictate science-based matching of recipients to donors, respect for the physiological limits of systems being operated upon, and strategic after-care aimed at reducing stress faced by vulnerable transplants from hostile environments (i.e. differences between present conditions and those previously adapted to). Our hypothesis predicts that equivalents to these principles can be applied in order to successfully translocate bulk “ecosystem” (an integrated whole rather than a collection of parts) from late-successional environments to earlier-stage ones. Where successful – due to wise donor-recipient site-matching, precise timing and placement of operations, and appropriate after-care through editing fine-scale habitat (microhabitat) conditions to more closely approximate the donor environment – ecosystem translocation offers unprecedented hope for accelerating succession and producing forest co-benefits by bypassing intermediate developmental stages. Our research explores this hypothesis experimentally by testing its specific predictions within a system of heritage hardwood forest and surrounding once-forested land, previously been for agriculture and aggregates but now undergoing rehabilitation and afforestation.

1.3 Experimental manipulations began at field locations near Duntroon, Ontario, in October 2017, with monitoring and analysis required through 2020. Here we report our progress and perspectives on answering both broad and specific research questions, from an interim position in the study. We follow an overview of: **(i)** research goals, rationale and hypotheses; **(ii)** experimental design and methods; **(iii)**

our the first fully-analyzed results and discussion, produced to a level suitable for academic publications and conferences (we shared these at international ecology symposia in August 2019), with section **(iv)**, a considered presentation of tentative conclusions, applications and planned steps forward for fully meeting remaining research goals. We hope the discussion offered in this final section will be of value even to readers unfamiliar with the scientific details in the preceding sections.

1.4 To test how well ecosystem properties typical of old spontaneous forests can be generated rapidly within environments undergoing rehabilitation or afforestation, we first needed to identify: **i)** which properties of ecosystems would be most salient to investigate; **ii)** a suitable donor forest (hereafter "D+") from which we could extract and utilize the "ecosystem" matrix of saplings, woody debris, organic litter and topsoil (including constituent plants, seeds and other organisms), which we collectively refer refer to as "living mulch" (hereafter LM); **iii)** "after-care" techniques for replicating donor forest microhabitats in a way that is both biologically effective and logistically feasible at industry scale; and **iv)** appropriate recipient environments for ecosystem transplantation trials.

1.5 Whether left alone or planted with trees, disturbed (including farmed) landscapes within forested regions usually regenerate forest cover over time, but pass through a predictable series of ecological stages (seres) in doing so. These include primary succession (ecosystem development from a position of no vegetation and no soil); stand initiation (ground conditions have evolved to support a moderate to high density of woody species); stem exclusion (the woody species grow tall and exclude most other resident vegetation through impacts on light and litter-layer conditions); understorey re-initiation (gaps in the canopy form due to weather disturbance, natural tree death, or management, and a diversity of new colonists with adaptations to mixed shade and woody debris microhabitats begins to establish); and old-growth (litter, woody debris and shade levels continue to shift and the best-adapted species produce the characteristic biotic communities of Ontario's primary hardwood forests at maturity. Areas licensed for extraction in Walker Aggregates' Duntroon quarry provided us with an exemplary sugar maple tract in this now-uncommon "old growth" class, to serve as the LM donor forest (D+) in our experiment. We expected that recipient environments at earlier stages of development would likely differ in their responsiveness to LM translocation and microhabitat modifications, depending upon which specific sere was treated. We therefore sought to include in our experiment recipient environments spanning a range of earlier seres, though restricted to environments consistent with landscapes under management by resource industries and occupying locations near enough to D+ meet criteria for both logistically feasible and ecologically responsibility (i.e. principle of good donor-recipient site-matching).

1.6 Under these constraints, we selected four locations within 6 km of D+ which each represented a different sere of forest development. S1 is a former gravel pit undergoing spontaneous primary succession for the past 25 years. S2 is a former farm field afforested with diverse native hardwoods to help offset biodiversity impacts of a new quarry. Just planted in 2015-16, S2 it is at a sere equivalent to stand-initiation. S3, a former field afforested with conifers ca. 1985, is approaching the end of the stem-exclusion sere. S4, with a similar history to S3 but planted ca. 1940, is well into the understorey re-initiation sere. To determine the extent to which outcomes of LM-translocation are attributable to shock from excavation or transport rather than stress imposed by new environmental conditions, we also included S5, a parcel of old growth forest contiguous with D+ but outside the limits of the new extraction. The core innovation of our experiment was to extract and transport truckloads of LM from D+ to S1-S5, in late autumn 2017 when vegetation had entered dormancy, and deposit the material within five large replicate recipient blocks (each 125 m^2 and piled to a depth of 0.3 m) at each sere. We have consistently monitored not-treated (NT) areas adjacent to each recipient block since before translocation, and we superimposed four comparative levels of a “microhabitat mimicry” treatment at each recipient block. We designed microhabitat treatment levels to determine experimentally whether simple structures capable of providing refuge from stressors in the new environment can increase the level to which D+ ecosystem properties emerge after LM deposition. In spring 2018, we left one quadrant of each recipient block alone (treatment level +LM) but translocated additional woody debris and replicated typical D+ microhabitat structures throughout the remaining quadrants. One of these was then also left alone (+LM+WD), while in another, we planted small shrub clusters (+LM+WD+SC). In the fourth quadrant, we erected artificial shade shelters (+LM+WD+SS), but only at seres without a resident canopy layer..

1.7 Although we measured several response variables of interest throughout 2018, we mainly viewed this duration as time required for ecological processes initiated by our treatments to fully take effect. We expected responses over the 2019 growing season to be the first reliable indicator of the outcome trajectories following LM translocation and microhabitat “after-care” strategies. We have therefore spent substantially more effort collecting data rather than analyzing it in 2019, and have to date only fully analyzed one piece of a much larger picture. The patterns produced by our initial analysis provide a template for what we may expect from other ecosystem responses, in part because the “property” we analyzed relates to multiple interrelated ecosystem components. Specifically, we have prepared a thorough analysis of the ground-layer vegetation community which emerges between first thaw and canopy-leaf out; this includes iconic spring ephemeral wildflowers, moss families, ferns, early summer herbs and more. The results shared here stem were derived from extensive statistical analysis of just a single level of ecosystem response, but over the coming months, similar comparisons among seres and treatment levels will be applied to a broad array of complementary 2019 data. This will include the vegetation patterns observed over the remainder of the 2019 growing season, but also a comprehensive suite of soil physio-chemical and biological properties, including meta-genomic barcoding of “eDNA” to assess the composition and functioning of soil bacterial, fungal and invertebrate communities. We will additionally evaluate an experimental assay of litter decomposition rates and mechanisms, explore thermodynamic indicators of ecosystem stress and adaptation, and more.

1.8 Our analysis of the number and identities of herbaceous and woody plants observed within 760 small (i.e. 0.25 m^2) sampling plots across all seres and treatments in spring 2019 revealed several factors that speak favourably on strength of our experimental system. This does not mean our hypotheses are all true, but rather that we have built an effective means to test them. First, translocating LM to S5 produced plant communities virtually identical to those in D+, indicating our method of extracting and transporting the material had negligible impacts on the (presumably dormant) biodiversity living within the LM. Departures from this pattern at other recipient seres can therefore be attributed to effects of the new environment rather than loss of LM viability during translocation. Second, every LM recipient block, regardless of sere or treatment, supported species compositions which were significantly more similar the D+ plant community than were adjacent NT areas. This alone is a major testament to the potential utility of ecosystem transplantation, but begs explorations as to the magnitude of similarity achieved, the meaningfulness of which species flourished or failed in which environments, and the likelihood that the observed communities are stable versus transitional. We inferred potential explanations for patterns of compositional differences among treatment and seres by subsequently analyzing variability in the numbers of different species (richness) occupying treated and NT areas of each sere. These ideas are hypotheses to be tested, through continued data collection and analysis, not conclusions. Third, some but not all of our microhabitat refuge structures had statistically significant effects, in some but not all of the recipient seres. The pattern of these differences is enlightening. Planting dogwood shrub clusters, for example, had no influence on the compositions of species which established from deposited LM, but installation of WD structures in S1-S3 significantly increased the compositional similarity of vegetation in LM-treated areas to that of the D+ ground-layer. Species supported included iconic woodland spring ephemeral species such as white trillium, wild leek and yellow trout lily. A trend towards similar effects manifested where we had installed artificial shade shelters in S1, and in S2, the positive effect of shade shelters on community similarity to D+ was strong and statistically significant.

1.9 Perhaps the most important result to emerge from out initial analysis is that the choice of recipient sere can have a much more dramatic influence on the outcome of LM translocation than any microhabitat alterations that managers could feasibly make. In S2, for example, which, despite abundant tree planting, still mostly supports tall grasses and aggressive agricultural weeds typical of old fields, NT areas expressed a similarity to the D+ community that was 14% of the score we had adopted as an “ambitious target for high similarity”. LM-treated areas expressed a similarity score 40% of the target where no additional WD had been added, a score 53% of the target where WD had been added, and a score 59% of the target where shade shelters were installed above the woody debris – all statistically significant differences. By comparison, translocating LM to the older plantations S3 and S4, in the absence of any other habitat modifications, produced plant communities expressing scores which were 77% and 81% of the high-similarity target (NT areas in the plantations expressed scores approximately 20% of the targets). From a goal of advancing ecosystem succession, it is difficult to judge whether “success” was greater at S2 or S3 (given the different background states, and the fact that both cases are successes in their own ways), but if the goal is to maximize the survival of LM biodiversity for conservation and industry-recycling purposes, targeting older plantations as recipients may be the wiser investment. The difference may become stark with time, as woodland species which initially established in the open seres S1-S2 may soon succumb to pressures from both the physical environment and the abundance of aggressive weeds. The latter factor may be most pressing, and its relative weakness in the older plantations (which support low vegetation abundance) may be the reason why LM-blocks in S3-S4 produced communities which were so similar to D+. Not only did diverse D+ vegetation emerge from the LM and tolerate the mixed-shade plantation environment, but the composition of the community was not diluted by an influx of colonists from outside the treatment area.

1.10 If results from our upcoming analyses and 2020 data collection resemble the interim findings we present here, the “transplantation surgery for ecosystems” hypothesis – including its principles of donor-recipient site matching, knowing and working within biological limits, and providing stress-reducing after-care by reducing microhabitat differences between past and present environments – may have pivotal utility in a range of increasingly important land management applications. The specific prediction that LM from a Niagara Escarpment maple stand can help produce, within nearby early-sere rehabilitation or afforestation sites, community compositions and other properties resembling the LM donor site has been partially verified. Discovery of different impacts at different seres, and these variably influence by microhabitat treatments, demands monitoring over a longer period and analysis of a broader spectrum of variables, as planned. LM applications may help produce much-valued co-benefits of old hardwood forests at depleted extraction sites and recent afforestations, but ecosystem features with the greatest similarity to donor forests are more likely to emerge after directing LM to older afforested environments with moderate shade, moderate clearings, and a low level of potentially antagonistic biodiversity present. Where overall higher diversity of species is and vegetation cover is a higher-priority goal than matching specific community compositions, LM may have an even wider range of utility, but benefits must be weighted against costs of source-depletion and likelihood of materials producing greater benefits if left alone, or applied elsewhere. When LM-type applications are targeted to young afforestations with old-field herbaceous cover, strong efforts may be required to prevent weeds form driving out target vegetation. Establishing larger-scale shade shelters may be the most efficient way to suppress undesired vegetation while simultaneously creating more favourable habitats for woodland species.

1.11 Extensive analysis of the roles played by different species and functional types, combined with investigation of the multidimensional soil-based components of the system, must ascertain the generality and mechanisms underlying the apparent support whish has so far been granted to our “transplant surgery” paradigm. Our final report at the end of 2020 will provide these missing links, but if the observed trends continue, the central implication for aggregate resource managers may be that they can maximize the biodiversity and habitat co-benefits of both site rehabilitation and offsetting programs. This would require comprehensively managing “living mulch” type resources such that fresh supply is available for application to strategic locations at strategic time points, including existing and planned afforestation projects – likely in combination with stand-thinning management – as they near the end of the stem-exclusion sere ca. 30-40 years after stand initiation.

2.0 INTRODUCTION

2.1 Background and rationale for the research

The need to better understand the ecology of forests and other ecosystems within human-dominated landscapes has never been more urgent, as scientists, governments, industries and public interests groups collectively seeks to combat what seems a legion of environmental crises: climate warming and skyrocketing variability; habitat destruction and fragmentation; biodiversity extinction; species invasions; and compromised ability of ecosystems to regulate essential processes such as the cycling of nutrients and water throughout landscapes. Aggregate producers have long played important roles in advancing and applying the science of ecological restoration, seeking to be good environmental stewards and adequately rehabilitate depleted extraction sites to other valued uses including naturalized areas which integrate well with the surrounding landscape. In the modern era, however, more is required than adequate patches for holes.

From a market perspective, producers seeking license to develop new aggregates resources are increasingly faced with challenges from both regulators and public advocates with respect to confidence that biodiversity, habitats and ecosystem functions associated with present landscapes can be replaced after extraction, particularly where quarrying below the water table of forested areas is required. The most promising solution to date has been to combine the best possible progressive site rehabilitation plans – which necessarily may include aquatic features where terrestrial ecosystems once reigned – with restoration offsetting. Restoration offsetting is the process of establishing ecological features expected to be impacted at a development site – including specific types of ecosystems, habitats, functions or biological communities – at a nearby offsite location which may otherwise be considered as exhibiting weak ecological functioning (e.g. brownfields, marginal-value agricultural fields). When offsetting is successful, it is usually because best ecological understanding is applied from project planning through implementation, long-term monitoring and adaptive management phases, and the restoration offsets are strongly protected from future land development. Failures, similarly, arise from poor ecological understand about how to control the development of desired ecological properties (such as a particular habitat or plant community) within environments generally fraught with physical and chemical stressor (e.g. poor soil quality, lack of protective structures to provide refuge against temperature and moisture extremes) plus critical differences in the biotic or “living” environment (e.g. colonization rates by “desired” versus “undesired” species). The biggest challenges to restoration offsetting arise, however, when the desired or “target” ecosystems spontaneously develop in nature over the course of centuries, but managers face pressure to replicate them at new locations over the course of decades, or sooner.

This is clearly a major problem facing worldwide momentum to increase forest cover in an ecologically responsible and sustainable manner, as go-to forestry methods of establishing quick-growing but homogenous conifer plantations have alarmed many ecologists for neglecting to foster the co-benefits of forests which are at least as important as carbon sequestration – namely, habitat provisioning and biodiversity conservation. The knowledge gaps are bigger for forest types which grow slower, and southern Ontario’s mixed and hardwood forests – including iconic Carolinian and Niagara Escarpment heritage stands – are among the slowest to develop. Land stewards who wish to replicate specific features of such forests – whether in the context of site rehabilitation, biodiversity offsetting, or contributing to broader regional afforestation initiatives – therefore face significant practical challenges in producing results fast enough to verify success within the lifetime of management windows.

Aggregate producers operating in hardwood regions therefore stand to benefit immensely if readily available engineering and restoration resources could be adapted into an efficient new method for rapidly producing forest habitats and biodiversity typical of spontaneous old-growth ecosystems within managed landscapes. Meeting ambitious ecological restoration and offsetting goals effectively and transparently can only increase public and regulator confidence in the sustainability of the aggregates industry and the crucial future role it may play in contributing broadly applicable environmental solutions to mounting ecological crises.

2.2 The RERAS study and team: Testing a new approach

We started research in April 2017 entitled the Rapid Ecological Restoration for Aggregates Sites (RERAS) study, aimed at closing the prevailing knowledge gaps which make hardwood forest restoration in southern Ontario so slow and uncertain to succeed. Our collective expertise within the University of Waterloo's Conservation and Restoration Ecology (CaRE) research group is world class on the topic of restoration ecology, in general (author S.M. is editor-in-chief of the academic journal Restoration Ecology, for example), and specifically we have advanced understanding applicable to quarry restoration and mitigation in particular (e.g. results of multiple research partnerships between P.R. and TOARC, spanning 2003-2019), while S.M. has multi-decade experience with forest restoration projects in Ontario and abroad. The perspective we bring to overcoming the difficulty of quickly establishing disturbed land on clear path towards ecological similarity with old spontaneous forests which are highly valued for their habitats and biodiversity provisioning is described here in relation to a "transplant surgery for ecosystems" paradigm. While the line of thinking is developed below, the chief implication is that resources sometimes available to aggregate producers as a bi-product of the lifecycle of resource development may be uniquely valuable in kick-starting the processes by which biological communities important to mature healthy forests begin to assemble within planted forests.

The specific methods we identified as having high potential to advance forest co-benefits in a rehabilitation or offsetting context were hypotheses that required rigours experimental testing, and windows into where best practices may eventually lie, if promoted through appropriate R&D pipelines. We attracted approximately \$440,000 in funding and critical donations of research-site access in order to carry out a 4-year study to evaluate the strength and practicality of the proposed strategy when applied in the field, thanks to TOARC, Walker Aggregates Inc., and The National Science and Engineering Research Council (NSERC)'s Collaborative Research and Development (CRD) grant program. Insightful improvements to the design and implementation of our experiment were provided by Ph.D. Candidate L. Jonas Hamberg, whose upcoming doctoral dissertation (expected in 2020) will founded in the three years of fieldwork he led within the RERAS system. Special gratitude is owed to contractor and collaborator Clearview Nursery Ltd. (Kevin Elwood), whose expert team worked tightly with researchers to efficiently complete all groundwork necessary to install and maintain the experiment, and whom donated access to one of our most valuable research locations (and additional thanks is owed to Clearview Township, for access to another site).

The funded research timeline required one year to properly install the experiment; a second for the relevant ecological processes to stabilize and begin to produce patterns which could be reasonably relied upon; a third year to intensively collect prolific data from multiple lines of response variables describing the state of the experimental systems; and a fourth year to make sense of all the patterns by evaluating which hypotheses best predicted the observed responses, adaptively direct appropriate follow-up fieldwork, and generate theoretical conclusions and best-practice recommendations – which inevitably call for new iterations of R&D. At the juncture of this interim report we are on the cusp of the third and fourth years, meaning a great deal of data has been collected but full analysis will still require several months, and the conclusions and applications suggested here are clearly tentative. Aspects of our results to date certainly encourage optimism, but the patterns we explore below should be viewed as hypotheses which we seek to refute, or not, based on the evidence yet to emerge from data which has yet to be analyzed (or collected, in the case of required 2020 fieldwork).

2.3 Succession-linked constraints on afforestation

Methods to rapidly produce biological structures and functions associated with mature spontaneous ecosystems through field manipulations have long provided somewhat of a "holy grail" for restoration ecologists; they would be extremely useful in a variety of contexts, but there are good ecological reasons why fully achieving such a feat will probably remain mythical. This is not to say that improvement over status quo approaches to forest-creation is impossible, but timespans substantially longer than human life are generally required for all of the interdependent ecological processes which eventually produce "old growth" woodlands to play out. Ecologists have long debated over the usefulness of various terms to describe the state of ecosystems which have transitioned from a long period of natural development and change to one of apparent stability – "climax state" and variations on forests being healthy, functional or well-adapted may be added to "old growth", in this respect – but they syndrome of features referred to is generally intuited reasonably by land managers. Part of the problem is that the course by which lifeless environments eventually produce ecological systems, and the trajectories on which the ecosystems grow, change and develop, is too frequently planned for as a simple, single process (called "succession") when in fact a diverse set of processes are acting and interacting in ways which can be intrinsically difficult to predict. It is because of the interactions and the uncertainty that there are so many sources for variable time lags in hardwood forest development.

The heart of succession theory is that organisms are constantly dispersing and resources in the landscape such as open ground or recently disturbed soils will eventually become colonized by pioneer species (including microbes, plants and animals), many of which have common functional of "life-history" traits including stress tolerance, rapid growth, reproduction and long-distance dispersal. Pioneer organisms inevitably change the environments they grow in, however, in ways which reduce the physical or other stressors experienced by subsequent colonists, including contributing to soil formation and ground cover by a variety of organic structures (living and dead). The resultant patchiness of resources and stressors tends to favour establishment and growth of different species with different traits than the pioneer wave, including more productive growth, increased competitiveness and eventually woody growth and higher tolerance of shade when young (or rather, species bearing such traits are the ones which are not "filtered out" by the surrounding environment as vegetation gets taller, casts more shade and sheds more organic litter). Not all environments produce soils capable of supporting tall trees, or remain disturbance-free long enough for such growth to occur, but in landscapes where such broader factors are suitable, woody stands of trees with pioneer-like traits tend to eventually established at high density (a successional stage, or sere, referred to as "stand initiation"). The shading and other environments alterations resulting from such stands growing quickly creates fine-scale ground-layer conditions (or "microhabitats") unlike anything that came before, and most residing vegetation cannot survive (reaching a peak at a stage called "stem exclusion", when there is minimal light, and biodiversity, on the floor of the nascent forest). Pioneer trees are naturally short-lived (for trees) and storms, forest usage, and other factors help transition the system into a third stage, called "understorey re-initiation", during which gaps in the canopy and heterogeneous light and litter conditions on the ground support emergence of new, diverse communities of plants and other organism, including specialists adapted to tolerate high shade, and others adapted to capitalize on patches or periods of lower shade (e.g. spring ephemeral wildflowers, such as wild leek).

2.4 Can forest succession be predicted, directed or otherwise controlled?

The mechanisms by which such understorey communities develop are some of the slowest and least predictable steps of forest succession, unfortunately, as they rely not only on particular confluence of habitat conditions (each of which depends on the rates at which other organisms grow, die and decompose), but the processes by which the appropriately-adapted organisms can arrive where they may flourish are complex (depending, for example, on factors such as the location, size and connectivity of potential source populations) and are themselves under constant pressures of change due to human activities (e.g. from global climate change to local development projects which may reduce source population habitats or erect barricades to successful immigration in suitable forests). The traits and identities of tree species producing the forest canopy evolve to reflect increasing shade tolerance and harder, slower-growing wood (capable of growing larger, and living longer) while the increased height of the canopy allows for growth of smaller trees and shrubs below which may develop into defined understorey layering. Increasingly, bi-products of the deaths of large trees drive changes in microhabitats and consequently the composition of species constituting the ground-layer (including, but not limited to plants), from standing deadwood structures to fallen limbs, trunks and stumps (collectively referred to as coarse woody debris) at different stages of decomposition, to the characteristic pit-and-mound ground formations produced from tree-fall. The culmination of such changes (and more) is the successional stage which has been referred to as "climax" and "old growth", but the complexity of factors involved and the roles played by specific legacies of history and location at different sites makes drawing precise boundaries around this final stage difficult.

Given the radical changes in biodiversity and ecological functioning associated with the transitions from stem exclusion through understorey re-initiation and the beginning of old growth, and the current dearth of such later-successional ecosystems in most human-dominated landscapes (in southern Ontario, for example, cover by such forests is less than 10% of what abounded prior to European settlement) , land managers seeking to maximizing contributions to offsetting industry or other impacts stand to make the greatest gains by producing, accelerating or otherwise facilitating such transitions in beneficial directions. Given the past century of forest planting and management in Ontario, and future prospects of widespread afforestation initiatives, total acreage of stands near the stem-exclusion stage is relatively high and therefore the scope of gains which could come from streamlining successional transitions that maximize forest co-benefits is great. The multidimensionality of factors which interact to produce such transitions in nature is very high, however, so the efficacy of interventions seeking to control just one or a few factors will likely be low. These constraints limit the utility of a traditional perspective in ecosystem restoration: that a damaged or degraded environment is like a good machine with a broken part, repairing the part will fix the machine.

2.5 Transplant surgery for ecosystems? A general hypothesis

Finding the origins of old growth to be too intricate, unpredictable and slow for managers to reasonably hope to replicate under typical project constraints, we propose an alternative analogy to the mechanical repair of ecosystems: rather than a pacemaker, perhaps what some ecosystems require is more like a heart transplant. We hypothesized that substantial biodiversity, functions and other valued properties of long-established ecosystems can be produced by managers within relatively recently disturbed environments provided that the collective, integrated products of such long succession are treated as a vital organ: difficult to build, but possible to transplant with appropriate knowledge, tools and care. In animals, organs are comprised of very different cell types which have nevertheless evolved to work in tight coordination and provide essential functions to a larger system. For ecosystems, although early-successional environments may function more like a collection of parts, the long biological legacy and specific networks of organism-environment interactions which produce old-growth ecosystems suggests the combination of ground-layer microhabitats, soil structures and biotic communities (including plants, microorganisms and animals) of a mature forest may be more aptly treated as a cohesive whole. Ecosystems with late-successional features in abundance are potential donors of such "vital organs" -- especially in areas which have been planned for land-use changes, like a patient in palliative care who has signed their donor card – while others, requiring restoration for whatever purpose, are potential recipients.

As with organ transplant surgery, successful ecosystem translocation would depend on strong respect for three principals: correctly matching recipient to donors; working within physiological limits of the system with respect to timing and implementing procedures; and providing after-care to minimize stress imposed on the vulnerable transplant, preventing rejection or failure. Stress for organ and ecosystem components arises from differences between the conditions being experienced versus those previously adapted to, and can therefore be alleviated by minimizing distinctions between new and old environments. This is one of the purposes of carefully planning donor-recipient matches, and with ecosystems, a potential recipient environment which is already on a likely successional trajectory towards similarity with the donor – due to the wider landscape, climate and species pool factors – would be a better match than a recipient on a very different trajectory. Respecting the matching principle would also mean consideration of ecological ethics and practices such as not moving organism beyond natural ranges, or putting vulnerable populations at greater risk. However, if the recipient was identical to the donor, it would not require the donation, so some stretching or exploration or possibilities is required. An environment on a trajectory towards a target state may not benefit from a transplant if it is still too different from the target, and it may not require a transplant if it is already close to reaching the target; the pertinent questions are therefore, what intermediate stages may require and benefit most from the intervention? If a valuable product has been donated, where should it be directed so that it does not go to waste? What are the limits on how well "after-care" can prevent failure of a less-than-perfect match? Respecting the principle of working within physiological limits of the systems would mean using strong knowledge of the ecosystem's physical and biological requirements on which to base precisely-planned excision, translocation and healing manipulations.

Finally, while after-care may include a variety options, we specifically envision efforts to increase the degree to which microhabitats in the recipient environment can provide refuge from new conditions which relocated organisms may be poorly adapted to. In forest restoration, this may include producing ground shade, woody debris and organic litter that mimics the ground environment of the donor forest – at least functionally, if not aesthetically. Researchers in other jurisdictions have experimented with "wholesale ecosystem transplantation" to mixed success, but these efforts have generally been in the spirit of progressive rehabilitation of surface mines, removing forest topsoil from areas to be extracted and depositing on areas just extracted (e.g. Vegetative Direct Transfer, or VDT, has been a successful technique for sand mine restoration in Australia; a similar spirit, thanks to Laurentian University ecologists, have guided successful re-greening initiatives of nickel-mined landscapes near Sudbury, ON). Lessons from such work are relevant, but success has mainly come in relative simple or quick-growing ecosystems (e.g. grasslands; boreal or tropical forests) and theory or experiments have not explicitly considered roles of donor-recipient matching, microhabitat mimicry as aftercare, or the very long succession time required in temperate hardwood forests.

2.6 Translocating living mulch and mimicking woodland habitats: Specific predictions

A general hypothesis is only useful if it can make specific predictions which can be tested or applied in systems which are available and of interest. Our organ transplant hypothesis does this for southern Ontario hardwood and mixed forests, such as those comprising the Niagara Escarpment World Biosphere Preserve, and the surrounding landscape which was almost exclusively comprised of similar types of forest cover prior to European settlement but which has since been converted to other uses including agriculture and limestone quarrying. In this system, best conservation practices are employed in areas designated for preservation, but some areas licensed for aggregates development support very mature sugar maple stands with rich native understorey communities and soil resources that may provide an exceptional “ecosystem donation” (prior to scheduled forest clearance and extraction of underlying aggregates, and as an alternative to long stockpiling of the material for later use as general “fill”). Potential recipients in need exist in surrounding areas which were originally forest but then farmed or mined, and have since been managed to meet site rehabilitation or afforestation goals. Here we refer to the integrated matrix of ecosystem products which are feasible to extract and transport as “living mulch” (or LM), consisting of vegetation propagules (seeds, but also living stems, roots and other tissues capable of regeneration), biotic communities in the soil (e.g. bacteria, fungi, invertebrates), organic and inorganic soil structures, woody debris and leaf litter layers.

Mature trees are of course essential components of old-growth forests, but they are unfortunately rarely amenable to surviving transplantation (like a skeleton). Their key roles of in shaping understorey habitat conditions combined with the potential absence of equivalent structures in recipient environments means that functionally replicating the missing microhabitat features should be main consideration in both prescribing after-care and in selecting recipients initially (e.g. an environment with some canopy closure or other strong shading agents, even if biologically distinct from the donor system, may offer better chances of success than any environment without such features). Saplings, shrubs and smaller-stature trees amenable to transplantation should be included as components of the LM, but with effort to excise and replant properly. Another implication of respecting the biological limits of the system suggests ecosystem translocation should be timed for when the ground in workable but most organisms living it have entered a state of dormancy (like sedation for a transplant patient), and excavated material should be excavated and transported immediately rather than stockpiled for later. Because woody debris structures varying in size, species composition and decay status play multiple different roles providing habitat refuges for woodland organisms, translocation after-care aimed at replicating such structures may reduce stress experienced by the LM biota and promote establishment of more characteristic forest communities. Although considerable work is required, these and other specific predictions of the transplant hypothesis are straightforward to test.

If the hypothesis is valid, then appropriately-targeted recipient locations at earlier stages of succession should begin to exhibit ecosystem properties more consistent with later-succession environments after being treated with translocated LM and woodland microhabitat installations. If treated areas in recipient environments express ecosystem properties which are no more similar to the donor ecosystem than are the properties expressed by untreated areas in the same environment, regardless of microhabitat modifications or the successional state of the recipient site, then the hypothesis can be refuted as untrue, or not useful. The particular experimental system we developed to test our predictions for Niagara Escarpment forests near Georgian Bay, ON, is the focus of the next section, while our first results and their implications are discussed subsequently.

3.0 EXPERIMENTAL DESIGN AND METHODS OVERVIEW

3.1 The Living Mulch Translocation Experiment: Donor forest and recipient seres

We designed a field experiment to methodically test **i)** the degree to which direct translocation of bulk living mulch (LM hereafter) from an old-growth donor ecosystem to more recently disturbed environments promotes therein the development of ecological structures and functions typical of the donor forest; **ii)** the degree to which the success of LM application depends on engineering of recipient environments to better reflect ground-layer habitat conditions in the donor ecosystem; and **iii)** the degree to which the success of LM application depends on the stage of successional development of the recipient environments

The source of the donor LM is a mature spontaneous sugar maple stand on the Niagara Escarpment near Duntroon, Ontario, which has never been cleared or planted in, and which supports diverse flora and fauna at the ground, shrub, sub-canopy and canopy layers typical of the region (a World Biosphere Preserve). It does not support any recognized threatened species and has been planned for clearance within a 40 ha zone that is licensed to accommodate the ongoing expansion of Walker Aggregate's Duntroon quarry. We hereafter refer to this LM donor location as D+. In addition to providing a source of bulk quantities of LM for experimental distribution to recipient locations at different stages of development (or "seres" of succession), unaltered areas of D+ provide excellent references for the types of plant communities and ecosystems properties which should be expected from a highly successful translocation operation.

We identified numerous potential recipient locations in the region and analyzed potential constrains and benefits with respect to each, in accordance with the described donor-recipient matching principles, settling on just four locations that should reasonably be included in our exploratory study. These shared a similar natural history to a point, with good reason to believe they supported forest cover very similar to D+ prior to European settlement; located less than 6 km from D+, each site is presently adjacent to forest contiguous with the larger complex housing D+ and presents an "upland" disposition. Post-settlement, the selected locations had been cleared for agricultural production, and then land-use history diverged in the manner we sought to test our predictions. At one location, gravel production supplanted agriculture; extraction operations continued for many decades but ceased in the mid-1990s and the site has been undergoing spontaneous primary succession since (i.e. in areas where extraction removed all soils but left unconsolidated inorganic substrates above the water table, and active rehabilitation was never carried out. We named this recipient locations S1, being the earliest sere to receive translocated LM.

The second selected location was used for agricultural crops and pasture until the current decade, when it was included in a progressive contemporary afforestation endeavour aimed at achieving a forest biodiversity offset to help mitigate expected ecological impacts from Walker Aggregates quarry expansion. The site was left fallow for two years then planted with a mixture of up to 20 different native hardwood and conifer trees in 2015 and maintained for two years with herbicide and irrigation applications, then left alone to become densely colonized by typical old-field herbaceous vegetation (including aggressive agricultural weed species) within and between the rows of planted trees (which produced less than 15% canopy cover in 2015). We evaluate the situation as similar to the stand initiation sere of classic forest succession models. As the second "youngest" recipient sere in the study, we named it S2.

The third and fourth recipient seres (S3 and S4) have many similarity to S2, having also been managed for conversion from agricultural land to forest, but the timing, goals and methods of the management differed. Both S3 and S4 are small conifer plantations, established using conventional forestry practices applied to patches of land where managers sought to prevent soil erosion. Using quick-growing conifer root systems to hold soils in place during drought and “blow sands” eras was the primary impetus for most early plantation forests in southern Ontario (and many later ones), with wood production a useful bi-product of good management practices (e.g. clearing rows to stimulate health growth). S3 had been established on property owned by Walker Aggregates (contiguous with the D+ and S2 locations) ca. 1985, primarily using white pine and white spruce mixtures, whereas S4 had been planted with red pine and white spruce in ca. 1940, on property currently owned by Clearview Township. No thinning or other stand management was ever applied at S3, and it is generally consistent with the stem exclusion sere of forest succession, though the areas we worked in were necessarily consistent with the end of this stage since applying and monitoring LM in a conifer plantation at maximum trunk and canopy density (peak stem exclusion) would be infeasible (and likely violate principles of reasonable donor-recipient matching). Management records for S4 are unavailable, but no thinning has been carried out in the past 30 years, many of the initially planted trees have died or produced a super-canopy while a tall sub-canopy of typical pioneer native hardwoods is approaching canopy status. The diversity and abundance of ground flora is moderate and S4 is overall consistent with the understorey re-initiation sere of forest succession.

In addition to the four recipient locations representing earlier stages of forest development, we included a fifth location, S5, which is at the same stage of succession as D+. S5 is superficially identical to D+ and completely continuous with it, but is located in an area of the Walker Aggregates property which will not be impacted by the quarry expansion. Translocating LM from D+ to S5 at the same time as operations at S1-S4 can help separate which aspects of the observed responses in S1-S4 are due to environmental differences from D+, and which are attributable to stress of the translocation procedure itself (i.e. organisms from D+ which fail to establish in S5 may not be amenable to transplantation anywhere). Additional information about D+ and S1-S5 may be found in Table 1; satellite and ground photographs are presented in Fig. 1 and Fig. 2, respectively.

Table 1: Niagara Escarpment field locations near Duntroon, Ontario, utilized in the living mulch (LM) and microhabitat translocation experiment

Code	Location name	Location type	Site description	Area (ha)	UTM (Zone 17T)
D+	LM Donor forest	Primary *Acer saccharum* stand	Primary upland (c. 450 m a.s.l.) *Acer saccharum* forest, with sections requiring clearing prior to licensed aggregate extraction; two such patches (N, S) were partially excavated to extract bulk LM (i.e. forest floor litter and topsoil layers) for immediate relocation to recipient seres in Oct. 2017	6.0	559709.00 m E 4915535.00 m N
S1	LM Recipient Sere 1: Primary succession	Disused gravel pit	Site of limestone sand and gravel extraction (c. 1940-1990) located < 7 km NE of D+; undergoing spontaneous succession, having never been rehabilitated	1.5	565109.00 m E 4919943.00 m N
S2	LM Recipient Sere 2: Afforestation at stand-initiation	Old field afforested in 2015	Native deciduous and coniferous trees planted on former farmland (< 0.5 km N of D+) to help offset clearing of D+; field underwent spontaneous succession for 5 years prior to planting with 20 tree species, which was followed by 2 years of irrigation and weed-control	6.0	559640.00 m E 4915723.00 m N
S3	LM Recipient Sere 3: Afforestation at stem-exclusion	Old field afforested c. 1985	*Pinus strobus* + *Picea glauca* plantation, established along edges and patches of the licensed extraction site (< 1 km NE of D+) that will not be extracted, and which required soil stabilization; not managed for wood production	1.5	559965.00 m E 4916232.00 m N
S4	LM Recipient Sere 4: Afforestation at understorey reinitiation	Old field afforested c. 1940	*Pinus resinosa* + *Picea glauca* plantation, established on former farmland < 4 km E of D+, to help stabilize soil; not managed for wood production	1.3	563981.00 m E 4916209.00 m N
S5	LM Recipient Sere 5: Old-growth forest	Primary *Acer saccharum* stand	Mature forest that is contiguous with and identical to D+, but outside the planned extraction zone (<0.5 km W of D+)	4.5	559439.37 m E 4915438.26 m N

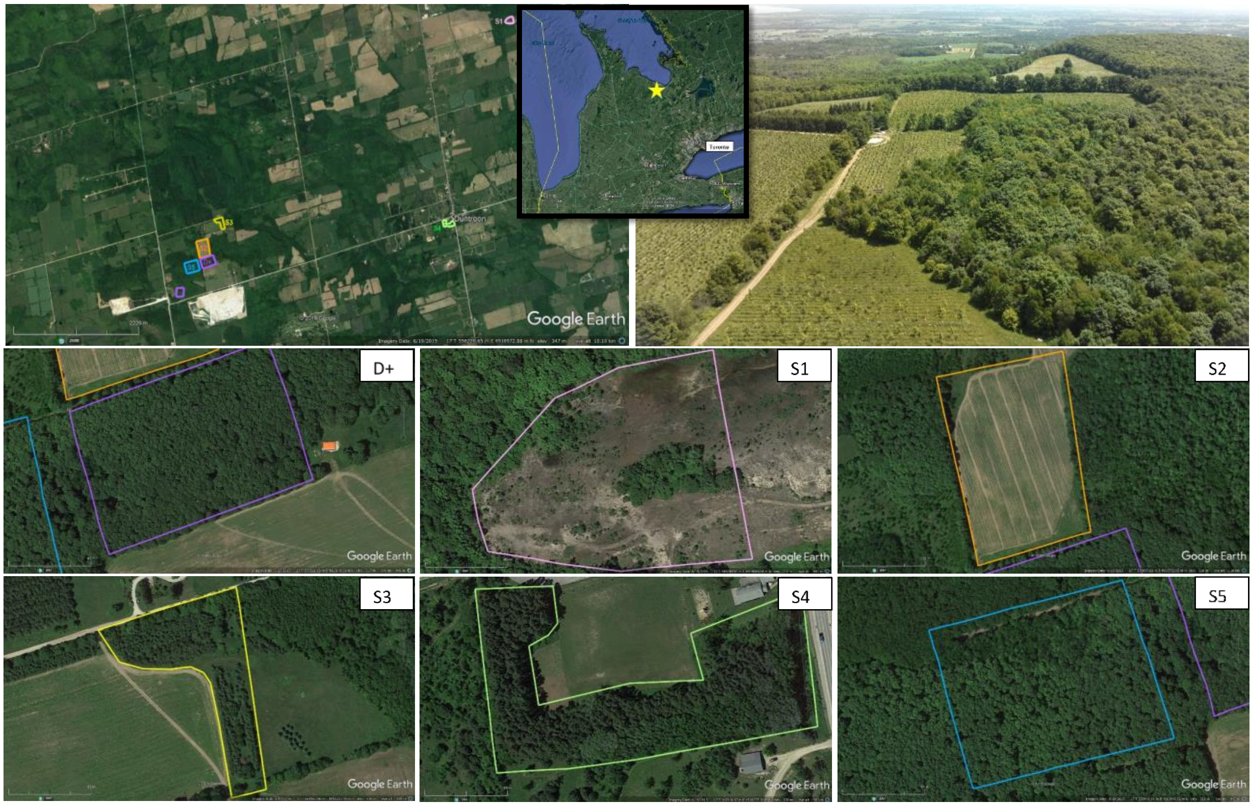

Figure 1. Overhead satellite imagery of the donor forest (D+) and recipient seres (S1-S5) incorporated into the living mulch and microhabitat translocation experiment (Duntroon, ON, Canada). Produced using Google Maps.

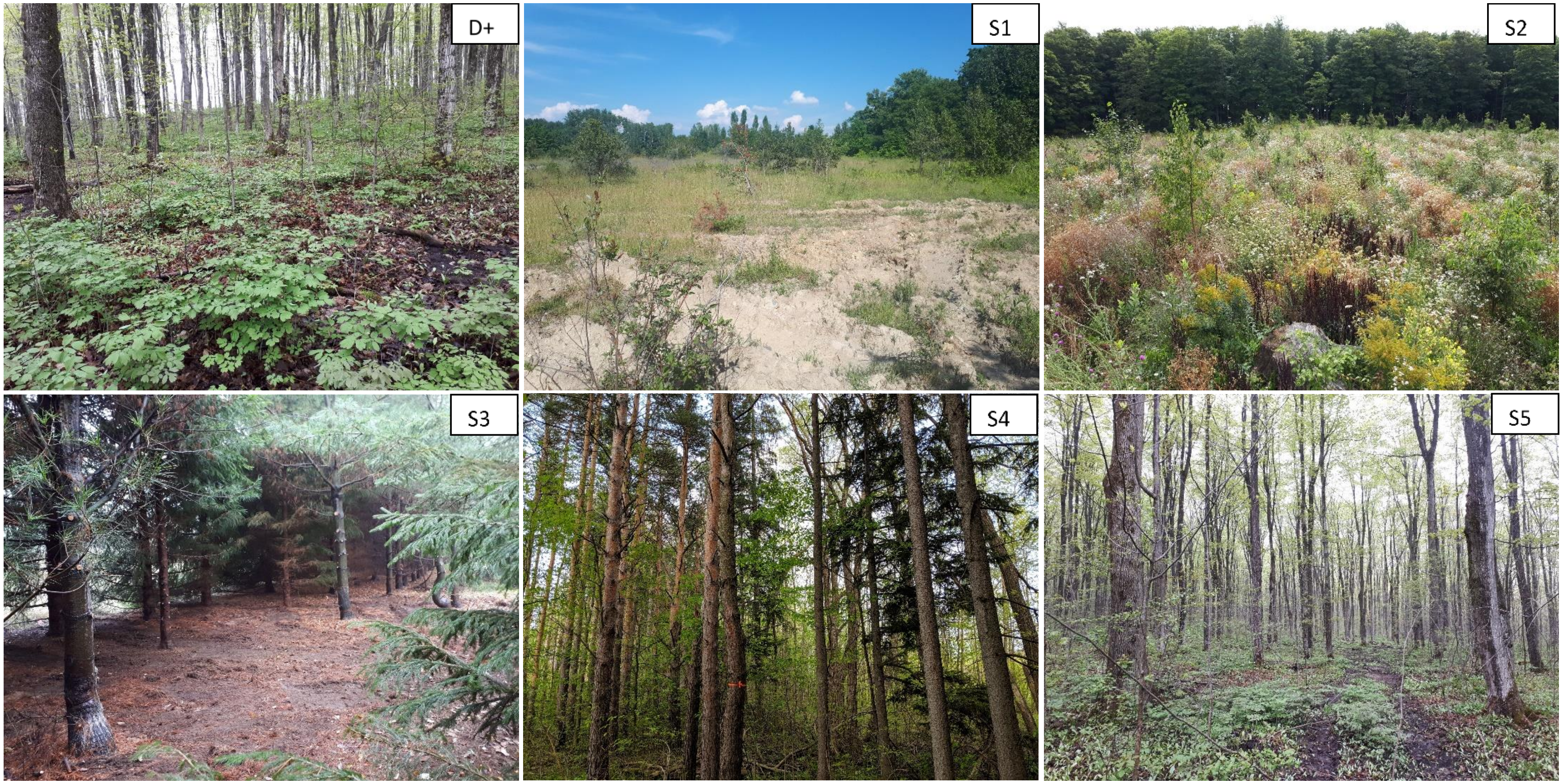

Figure 2. Donor (D+) and recipient (S1-S5) seres incorporated in the living mulch and microhabitat translocation experiment (Duntroon, ON Canada).

3.2 Applying the living mulch (+LM)

We began installation of the experiment at the end of October 2017, after most vegetation had entered states of dormancy for winter but before winter weather could interfere with operations. The work consisted of progressively extracting LM from selected zones within D+ using light excavation equipment and transporting truckloads of the material to recipient blocks at S1-S5 on the same day as extraction. The excavated LM included forest floor organic litter layers (leaf litter, small (fine) woody debris, some larger (coarse woody debris) plus the underlying 30 cm of topsoil, [including inherent vegetation, propagules (e.g. seeds, spores, clonal roots), micro-fauna and microbes]. We salvaged 1-3 m tall saplings with intact root balls during excavation and hand-planted them in the freshly-deposited LM at recipient blocks (ca. 5-7 saplings per block). Each recipient block consisted of a 12.5 m x 10 m areas which was cleared of major obstructions (including mowing and herbicide application for the dense herbaceous cover at S2) and then filled with a 30 cm deep layer of LM (including working around existing trees). To facilitate the logistics of LM application, recipient blocks in the wooded sites were necessarily created within areas that included small natural clearings more than dense tree cover. Within S2, recipient blocks were established at five locations within a field which had been planted with trees in 2015 and at five locations within a field planted in 2016 (only the 2015-planted blocks were included in the analyses shared here). At each of the other recipient environments (S1, S3-S5), we installed five replicates of the LM recipient block. In total, we installed 30 LM recipient blocks, each corresponding to 125 m^2 of ground area.

3.3 Creating microhabitat refuge structures

To test how effects of LM deposition depend on the creation of additional microhabitat structures consistent with the donor forest, we subdivided each of the LM recipient blocks into four equal quadrants (6.25 m x 5 m) and in spring 2018 imposed the following four levels of our microhabitat refuge treatment. A summary of the treatment levels may be found in Table 2. In one of the quadrant areas in each recipient block, we did not impose any additional changes after depositing the LM; we refer to this level as +LM.

3.3.1 Translocating additional woody debris (+LM+WD)

To the remaining three quadrants in each block, we introduced approximately 10-15 m^3 of additional woody debris (hereafter WD) which we had extracted from D+ in the spring following LM translocation – mainly as fallen trunks, limbs and old stumps spanning a range of sizes and states of decomposition. We distributed the WD sporadically throughout each area to create small piles and other structures resembling natural woody debris formations in D+. In spontaneous old-growth forests, such formations provide valuable physical variability, or heterogeneity, with respect to a number of ground-layer environmental conditions crucial to plants and soil organisms, including light intensity, heat, humidity, and biochemical products of wood decomposition. In each recipient block at each site, we made no further alterations to at least one of the three quadrants treated with additional WD and refer to this treatment level as +LM+WD.

3.3.2 Planting dogwood shrub clusters (+LM+WD+SC)

In a second WD-treated quadrant, we planted a shrub cluster (SC) of six dogwood saplings (*Swida alternifolia*), each 1 m tall, in a circular formation approximately 1.5 m in diameter. Alternate-leaved dogwood is a quick-growing native hardy to a range of light conditions and is a common component of local hardwood forests consistent with developmental stages ranging from understorey re-initiation through old-growth. The woody growth varies in height from 1-2 m tall shrubs to 4+ m small trees, contributing variably to ground-shading and facilitating ground-layer biodiversity. The after-care principle of the transplant hypothesis predicts if that shading and other impacts of the growth of dogwood clusters make local environmental conditions more similar to D+, more biodiversity that is characteristic of D+ will establish where LM is near an SC. We refer to this treatment level as +LM+WD+SC.

Table 2: Five levels of habitat refuge creation within the Living Mulch relocation treatment

Code	Level	Microhabitat treatment applied	Test of effect
NT	1. No Treatment	None; refers to areas within recipient locations which did not receive relocated materials or other alterations. Sampling of NT was carried out 10-20 m adjacent to the installed Living Mulch Recipient (LMR) plots.	-
+LM	2. Addition of Living Mulch	Living mulch (LM) was excavated in patches from donor forest floor (e.g. upper 30 cm of topsoil, propagule banks, litter layers, etc.) and relocated immediately in bulk to recipient locations (Oct. 2017). Translocated materials were deposited evenly onto 12.5 m x 10.0 m patches of ground, to a depth of 30 cm, working around existing trees but otherwise completely covered resident vegetation and surface features. One quadrant was designated "+LM" and assigned to receive no further alterations.	+LM *vs.* NT
+LM+WD	3. Addition of Living Mulch and Woody Debris	Pieces of dead, fallen trees (e.g. stumps, trunks, limbs) varying in size and decomposition state were collected from the donor forest and relocated to the LMR plots (May 2018). Approximately 15 m^3 of woody debris (WD) was distributed evenly throughout the three remaining quadrants per plot. One quadrant was designated "+LM+WD" and assigned to receive no further alterations.	+LM+WD *vs.* +LM
+LM+WD+SC	4. Addition of Living Mulch, Woody Debris and Shrub Cluster	Six *Swida alternifolia* nursery-stock saplings (1 m height) were planted in a 2 m diameter circular shrub cluster (SC) near the centre of one quadrant which had received WD, in each LMR plot (May 2018).	+LM+WD+SC *vs.* +LM+WD
+LM+WD+SS	5. Addition of Living Mulch, Woody Debris and Shade Shelter	A shade shelter (SS) was constructed near the centre of the remaining quadrant which had received LM and WD, in each LMR plot at locations S1 and S2. Each structure consisted of cedar fence posts installed in the four corners of a 4 m x 4 m area, and cut to a height of 1.8 m. A sheet of black horticulural shade cloth, able to reduce peak incident solar radiation by 70%, was spread across the tops of the posts and used to cover the ground below (May 2018; tarps removed and reinstalled seasonally).	+LM+WD+SS *vs.* +LM+WD

3.3.3 Constructing artificial shade shelters (+LM+WD+SS)

The last type of environmental modification we applied was also aimed at increasing ground shading to increase the similarity of the LM biodiversity "yield" to D+, but designed to dodge the potential ecological vagaries of living shading agents, which could fail to survive, or produce meaningful canopy in a useful amount of time, or negate beneficial impacts on neighboring organisms by out-competing them for nutrients or water. We therefore erected artificial shade shelters (SS) within the remaining WD-treated quadrant. Due to logistical infeasibility, as well as likely lack of need, we did not attempt to construct shelters within S3-S5 recipient blocks because they already experienced a mostly-closed canopy. We built each SS using four peeled cedar posts (12 cm diameter), installed to a height of 1.8 m in the corners of a 4 m x 4 m area which was located near the centre of each designated quadrant. We then attached across the tops of the posts a removable tarp made of black horticultural shade-cloth which was manufactured to reduce incident solar radiation by 70%. We extended the tarps in early summer and took them down again in late autumn corresponding to canopy leaf-out and shedding in D+, beginning in 2018 and planned to continue through 2020. We refer to this treatment level as +LM+WD+SS).

3.4 Required controls: Not-treated (NT) areas at S1-S5

We have designed this research to test predictions of the organ transplant hypothesis by carefully comparing the ways in which ecosystem properties expressed within the LM recipient blocks are similar to, or different from, properties typically observed within D+. Such a comparisons are only meaningful, however, if we also have a reference for what ecosystem properties are like in the areas of recipient seres which did not receive any LM. From a practical perspective, it would not be worth the work of translocating LM if the resultant ecosystem were no closer to D+ than areas which had been left alone. We therefore included "not-treated" (NT) areas, generally 5-10 m adjacent to the LM recipient blocks, which we are monitoring using all of the same measures as in the treated areas, and including in analyses for crucial tests of whether LM application produced target properties better than taking no action. We refer to this treatment level as NT.

3.5 Ecosystem responses: What we are measuring, and why

The design of our experiment permits direct comparisons among similar environments at different stages of development as they respond to our alternative sets of management interventions. We are testing predictions that different interventions will produce different degrees of similarity to the ecosystem properties characterizing D+, and so the scope of ecosystem properties we chose to measure has major implications for what patterns we may be capable of finding. Selecting an optimal suite of response variables which balances comprehensive knowledge with feasibility and expense, we are presently monitoring responses in the following categories. One aim of our analysis will be to determine how different responses are related to each other, and whether a narrow subset of responses may indicate the status of others, or otherwise predict states of the broader system.

3.5.1 Plant communities

We have prioritized assessing the plant communities growing at the study locations as the best immediate-term indicators of ecosystem status because ecology has well-developed tools for drawing inferences about the structure, functioning, and development of ecosystems by considering multiple related aspects of plant community organization. Metrics including the total number of plant species, their taxonomic identities (e.g. family/genus/species), relative amounts of ground covered and predominant growth forms (e.g. moss/fern/forb/grass-family/woody vine/shrub/tree) can be important in this regard, as can the biogeographic origins of the species (e.g. native or exotic), life-history strategies (e.g. opportunist vs. strong competitor) and relative rates of change in many of these properties over time or space. We have been surveying plant communities in different ways for different purposes within the experiment, with our analysis of the spring 2019 vegetation communities our primary source of insight into results thus far.

3.5.2 Soil physio-chemistry

Comprehensive analysis of physical, chemical and biological features of the various manipulated and resident soils in this experiment will likely tell us as much, or more, about the effects of our treatments as will the plant communities. Soil conditions and dynamics can both control and correlate with other aspects of ecosystem development which are important in their own right but which are also especially relevant due to interactions with vegetation. We have collected and stored more than 1000 strategic soil samples from different treatments and time points in the experiment, and have planned extensive physio-chemical laboratory analyses for winter 2019-20 in collaboration with SGS Guelph, including quantification of soil pH, P, K, Mg, Ca, Zn, Mn, Cu, B, Fe, ammonium, nitrates and organic carbon.

3.5.3 Soil biology and "eDNA"

To directly assess which organisms are living within which soils, and the nature of the functions they are contributing to, we are opening a new toolbox that has recently been developed on the cutting edge of molecular biology. Named meta-genomic barcoding of environmental DNA, or "eDNA meta-barcoding", this approach isolates and amplifies tiny fragments of DNA present in soil and enables identification of microorganisms (sometimes with species numbers measuring in the tens of thousands) living therein (or rather, within the previous 6 months, given time required for DNA from dead organism to degrade). We plan to expose subsets of our collected soil samples to so-called "shotgun" eDNA exploration, in order to characterize and compare treatment levels and seres with respect to soil bacteria/archaea; fungal networks; and potentially invertebrates including insects, arachnids and earthworms (each requiring different methodological approaches and expenses). The specific laboratory methods are complex, technologically demanding, and still in their early stages of development, but this enables the RERAS to contribute to a frontier of environmental biology while the new techniques help us meet our primary research goals. Collaborating with academic and commercial partners with the necessary expertise will be critical to success on these fronts and to this end we are exploring partnership with two young companies incubated from the University of Guelph's Barcode of Live Initiative: AgriSeqSolutions and Precision Biomonitoring. We are planning for initial genetic sequencing and analysis of soil fungal and bacterial communities over winter 2019-20, with insight into the biology of the late-2019 soils expected by spring 2020.

3.5.4 Decomposition rates and mechanisms for leaf litter in soil

We are paying close attention to both the physio-chemical and biotic properties of soils in our experiment because these can interact to drive soil processes foundational to ecosystem development, such as how plant biomass produced by the system is subsequently decomposed and recycled through food webs to influence soil organic matter amount and quality. The rate at which leaf litter decomposes, and more specifically, the different rates at which carbon versus nitrogen components are metabolized by decomposers in the soil, can impact different aspects of forest functioning (including below-ground carbon storage) and indicate how well different pathways of decomposition are operating. The level of matching between LM-treated areas and D+ with respect to decomposition dynamics cannot be inferred from chemical or genetic analysis of collected soil samples, however. Instead, we began an experiment-based assay in fall 2018, in which we collected freshly fallen leaves within the donor forest, dried them, and portioned the material into several hundred 5.00 g samples. We then transferred the weighted samples to custom-sewn nylon mesh pouches and buried these shallowly, in replicate clusters of 4, throughout D+ and S1-S5.

The mesh size of the pouches allows the most common decomposition agents (bacteria, fungi, invertebrates) access to the dried leaf material but keeps the undecomposed leaf remnants intact so that, after a designated period, the samples may be retrieved, re-dried and re-weighted to determine how much of the sample decomposed per unit of time. Elemental analysis of the material (plus control samples which had never been buried) helps determines the relative rates of decomposition of carbon versus nitrogen compounds in the leaf biomass, from which inferences about bacterial versus fungal decomposition pathways can be made. The ability for the mechanisms of litter decomposition to resist and recover from the stressors of translocation and new environmental conditions will help indicate how well the “living” parts of living mulch maintain functionality in the applications we are testing. We buried all litter pouches in early spring 2019 and retrieved the first set of samples for analysis in late autumn 2019, with remaining replicates to be retrieved in 2020. Analysis will proceed over winter 2019-20, with first insights into decomposition functioning expected in spring 2020.

3.5.6 Ecosystem thermodynamics

We seek to understand fine-scale ecological mechanisms because knowing how they work can help us understand and forecast ecosystem responses to our treatments over larger scales of space and time. This requires discovering the nature of the linkages among phenomena observed at different scales – for example, the ways in which the interactions among species and environments influence an ecosystems broader capacity to remain stable in the face of large climatic fluctuations. Recent engineering innovations have enabled ecologists to begin studying potential indicators of broader ecosystem status (such as stress levels experienced, or current development stage) related to the thermal properties, including the proportion of incoming radiation that is used by organism to do work, which can be inferred from the amount of heat radiated from the system. Using high-resolution thermal cameras mounted to a remote-operated aerial vehicle, we have been repeatedly surveying thermal properties a subset of the treated areas (S1 and S2), in collaboration with colleagues in the University of Waterloo’s Engineering Department. One purpose is to help determine the potential use of this technology for monitoring the progress of ecological restoration, while another is to gain mechanistic insight into relationships between ecosystem structures and functions in our system, including thermo-regulatory properties pertinent to climate change resilience and adaptation. Work on this front comprises a substantive component of Ph.D. Candidate Hamberg’s doctoral dissertation, and analyses of 2019 thermal data will be available to share by spring 2020.

4.0 INTERIM RESULTS & DISCUSSION:

Translocating mature forest floor and constructing woodland microhabitats can initiate establishment of late-successional plant communities at early-successional afforestations

A key challenge and goal of this research is to generate, within a 4-year research window, reliable and useful new knowledge about how to control the trajectory of ecosystem development – even though the actual developmental processes we hope to influence typically require from decades to centuries to play out. Our transplantation surgery paradigm predicts ways in which this may be possible, yet time is still required to not only do the work of installing, maintaining and monitoring experiments, but more importantly, to allow for the potential ecological outcomes of the treatments to become fully realized, whatever those outcomes may be. For this reason, although we monitored various ecosystem responses and conducted preliminary analyses throughout 2018, we place greater stock in the patterns which resolved from surveys completed in 2019. These show the status of plant communities and other indicators as they occurred at least one full year (an important duration in temperate ecosystems) after initiation of all treatment levels. We expected the sharpest potential transitions in community structure and linked ecosystem properties – new colonists establishing securely and beginning to flourish, for example, or translocated species succumbing to local stressors or competition and becoming extirpated from their communities –to occur over the first full growing season and winter in a “sink or swim” year. We fully expect still further changes to come, but given an overall trend of similarity between the 2018 and early 2019 community patterns, we are confident the data at hand represents non-spurious responses by the system to the imposed treatments.

We will begin comprehensive analysis of the full set of vegetation surveys collected throughout 2019 over winter 2019-20, but the one analysis which have carried out to a standard of completion suitable for peer-reviewed academic journals – based on plant community data collected in May-June 2019, has produced intriguing and optimistic results. Much of the biodiversity inhabiting mature hardwood forests such as D+ is associated with spring ephemeral plant communities. These are comprised of suites of ground-layer herbaceous species which have evolved to carry out the majority of their yearly growth in the brief period between spring thaw and full leafing-out of the forest canopy in early summer – a life-history strategy called shade avoidance, rather than shade tolerance. We expect such species may have higher tolerance for the earlier-sere recipient environments than others in the LM matrix, and be valuable in applications. In our spring 2019 survey we were able to examine communities including many spring ephemerals within D+, and assess whether there was evidence for the establishment of similar communities within treated and not-treated areas of S1-S5. We also observed, shortly after their emergence, many plants typically comprising the summer flora in the ground-layer, indicating analysis of the spring data would likely grant a fair preview of at least some of the structure of the later-season community as well. One impetus for priority analysis of the early-season data gain insights which we could share with academic colleagues at conferences in August 2019. We accomplished this, receiving helpful peer feedback as well as accolades, through contributed oral presentations at the Annual Meeting of the Ecological Society of America in Louisville, KY ((12 Aug 2019, author S.M. presented), and at the Canadian Society for Ecology and Evolution conference in Fredericton, NB (21 Aug 2019, P.R. presented).

Full details of the analysis are beyond the scope of this report (though will be published in the academic literature) but our insights came after devising a fair and accurate way to score each small plot of vegetation (0.5 m x 0.5 m) sampled in recipient seres (S1-S5) with respect to how similar its composition of plant species is to that typically observed within a similar-sized patch of donor forest (D+). In the spring 2019 sampling, from May 15 through June 8, we identified every species of fern, herb, grass, vine, shrub and tree possible (plus commonly reoccurring moss families) within each of four 0.25 m^2 plots placed at random positions within each of the differently-treated quadrants in every LM recipient block. This added up to 60 small sampling plots per site where no shade shelters had been installed (S3-S5), and 72 plots per site where they had been (S1-S2). At each sere we also sampled 60 plots within not-treated (NT) areas surrounding the treated areas, for a total of 500 sampling plots across all treatment levels (NT; +LM; +LM+WD; +LM+WD+SC; +LM+WD+SS). We characterized the plant community composition within the donor forest by assessing 160 sampling plots.

4.1 Using the Jaccard index to calculate compositional similarity (Jsim) among plant communities

For each plot in S1-S5, we quantified its average similarity to the plots sampled within D+ using the Jaccard index, a mathematical tool also known as Intersection over Union which is used in many disciplines to compare pairs of sets with respect to the proportion of elements that shared between sets versus unique to one or the other. The index ranges between 0.00 (no elements in common between sets) to 1.00 (both sets identical). We defined the compositional similarity to D+ of each sampled plot in S1-S5 as the mean of the values calculated when comparing that plot to every plot sampled in D+. This metric (termed "*Jsim*") is useful because it inherently accounts for the natural spatial variation of the plant community within D+. Even if LM application "worked perfectly", there is no single idealized group of species we would expect to find on any particular patch of ground. We would, however, expect such a patch would support some set of species that is more consistent with the full group of D+ reference plots (N=160) than would a patch of early-succession ground that did not receive any treatment, or that received a treatment which worked less than "perfectly".

We set an ambitious bar for success using this evaluative approach by assuming that the calculated *Jsim* value for a sample plot where LM application was maximally successful would be approximately the same as the average degree of similarity between any one small plot in D+ and the full set of D+ reference plots. We calculated Jaccard similarity between each D+ sample plot and the full set of D+ sample plots separately, and took the mean of these values as our bar for "high similarity to the D+ plant community". The most successful possible outcome of LM application would therefore be indicated if plots sampled in areas which received LM expresed similar index values to this D+ reference value while plots sampled in adjacent NT areas expressed lower *Jsim* values which are substantially lower.

4.2 Testing predictions by comparing outcomes among treatment levels

We analyzed the set of *Jsim* values for all plots sampled at recipient seres in spring 2019 as a function of the sere (S1-S5) in which the plot was located, and, as a nested effect within that sere, which microhabitat treatment had been applied at the sampled location (i.e. NT, +LM, +LM+WD, +LM+WD+SC, or +LM+WD+SS). Using generalized linear model analysis, we also included random effects to account for potential autocorrelation among plots sampled within the same recipient block (or surrounding area), and made previously planned statistical contrasts between different, specific treatment pairs to test predictions. The test for the prediction that LM application would increase the similarity of S1 vegetative composition to that of D+, for example, is to determine if *Jsim* for plots in LM-treated areas of S1 is statistically greater than *Jsim* for NT plots in S1. To test the prediction that adding extra WD microhabitat structures would induce an even greater degree of similarity to D+, it is necessary to contrast *Jsim* in +LM+WD plots against *Jsim* in the +LM plots. Similarly, the +LM+WD treatment level must serve as the baseline for comparison when determining if yet greater similarity to D+ is produced by installing shrub cluster (SC) or shade shelter (SS) modifications, as these different refuges against excessive solar intensity were only applied to areas that had received WD in addition to LM.

4.3 Vegetation responses in the donor forest (D+) and not-treated (NT) areas of recipient seres

Both the effect of sere and that of treatment nested within sere were highly significant, statistically (i.e. p-values < 0.0001), as were the results of some (but not all) of the tests of predicted effects. The reference D+ value for *Jsim*, quantifying the average compositional similarity of each sample plot in D+ to the full set of D+ sample plots, was 0.177 (+/- 0.005). As discussed, we adopted this value as our "ambitious" target for high similarity to the donor forest. By comparison, mean *Jsim* for NT areas of S1 (the former gravel pit) was 0.013, or 7.5% of the high-similarity target. The NT areas of S2 (afforested in 2015) supported plant communities which were approximately 14% of the target value, while NT zones in S3 and S4 (afforested with conifers in 1985 and 1940, respectively) expressed mean *Jsim* values which were 23% and 19% of this target, respectively. In S5 (mature maple stand nearly indistinguishable from D+), the mean *Jsim* value was technically 104% of the target value, but this difference from 100% of the target value was not statistically significant. This pattern indicates that plant communities spontaneously residing in even the earliest successional environment investigated share some species in common with D+ (mean *Jsim* would be 0 otherwise) but not many. It also shows that the degree of similarity to D+ increases with afforestation and time, but not my much: even 75 years after tree-planting, the composition of understorey vegetation was less than 25% of the high-similarity target value. The complete similarity to D+ exhibited by S5, by comparison, indicates that S5 is a suitable location for serving its function as reference environment, representative of the D+ conditions but protected from future impacts of the quarry expansion.

4.4 Similarity of plant communities in LM-treated areas to D+

4.4.1 Responses at S5

Knowing the pattern of similarity to D+ vegetative composition in NT areas of recipient seres sets the stage for evaluating the effects of LM application and woodland microhabitat structures. First, mean *Jsim* in +LM areas of S5 was 103% of the target value, and not statistically different from NT sections. This indicates that our method of extracting and translocating the LM likely had negligible impacts on the vegetation residing within the LM. Failure of +LM to produce similar values at other seres should therefore be attributed to environmental conditions at those locations, not to the mechanical stress of translocation itself. The +LM+WD areas of S5 produced *Jsim* values which were 110% of the high-similarity target (not statistically different from +LM), indicating plant biodiversity within LM may not require or benefit from additional WD within the late-successional environment, but it was not harmed by our manipulations, either. The areas of S5 treated with both woody debris and dogwood shrub clusters (+LM+WD+SC) supported plant communities expressing *Jsim* not different from +LM+WD, also indicating no benefit or detriment of the microhabitat manipulation within the mature forest.

4.4.2 Responses at S1-S4

Introducing LM to the four earlier seres produced plant communities expressing *Jsim* values which were less than those at S5, but significantly greater than values for the corresponding NT areas at each sere. In S1, *Jsim* for +LM plots was 55% of the target high-similarity value, compared to 7.5% for NT plots. In S2, *Jsim* for +LM plots was 40% of the target, compared to 14% at NT areas. The *Jsim* values for +LM areas at the two older plantation seres are greater still. In S3, +LM areas supported plant communities expressing *Jsim* values which were 77% of the high-similarity target, whereas in S4 the value was 81%, compared to 23% and 19% in NT areas, respectively. This pattern of relationships, along with the effects of microhabitat structures discussed next, is illustrated in the graph below (Fig. 3). It is perhaps just as clearly present in the photographs which follow, of exemplary locations within S1-S2 (Fig. 4), S3-S4 (Fig. 5), and S5-D+ (Fig. 6).

4.4.3 Effects of translocating additional woody debris (WD)

Translocation “after-care”, aimed at increasing the degree to which shade and woody debris microhabitats at recipient blocks resembled D+ conditions, incurred different effects at different seres. Introducing additional WD at S1 produced communities expressing *Jsim* 64% of the high-similarity target, compared to 55% at +LM plots, but this improvement was not statistically significant. In S2, however, a similar increase – from 40% to 53% of the target value, when comparing +LM to +LM+WD – was statistically significant ($P<0.005$). In S3 also, addition of extra WD to LM-blocks produced a statistically significant increase in *Jsim*, from 77% to 86% of the high-similarity target. In S4, plant communities in both +LM and +LM+WD areas expressed the same *Jsim*, a value 81% of the high-similarity target.

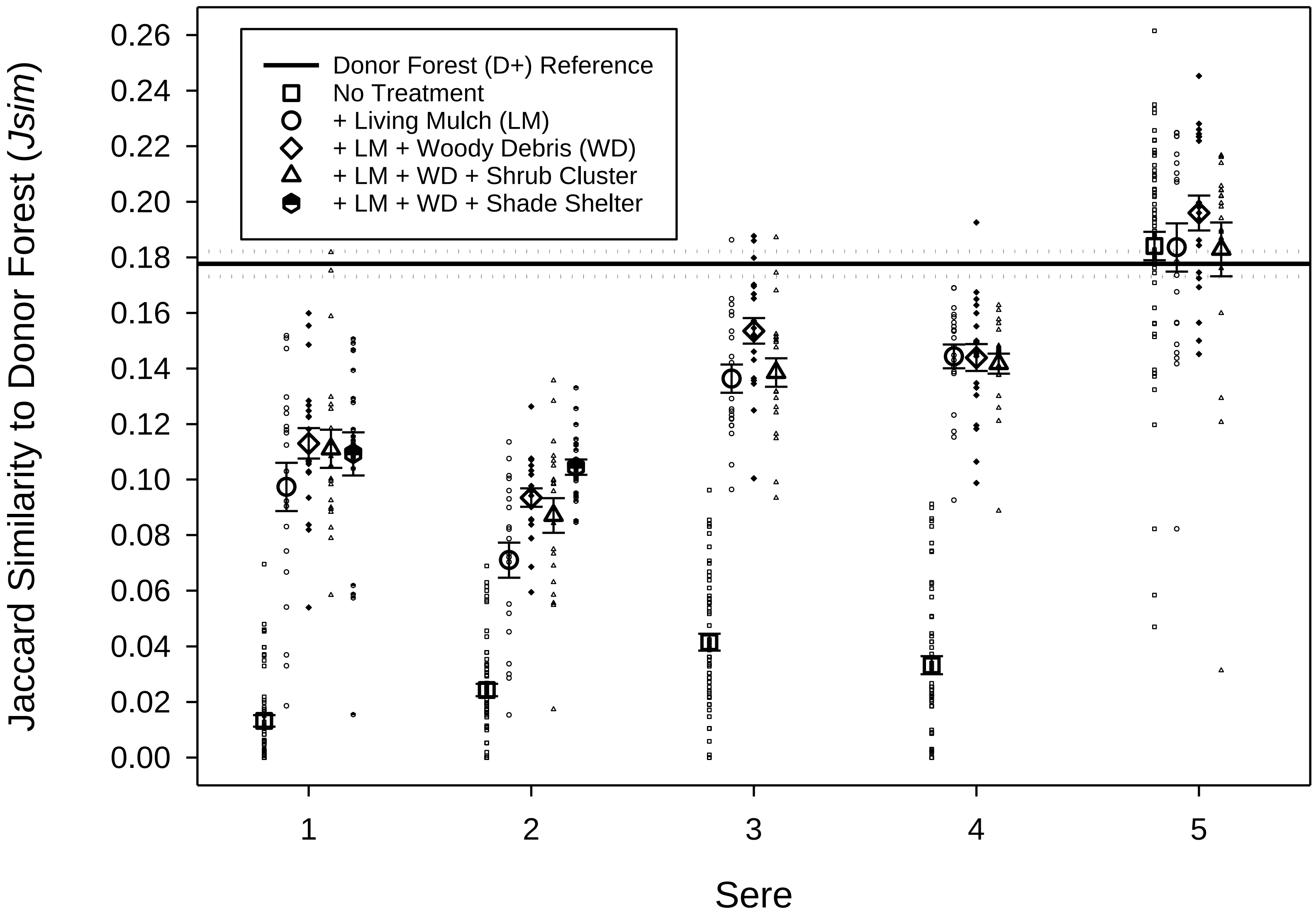

Figure 3. Responses of *Jsim* to recipient sere identity, and the living mulch translocation treatment levels nested within sere identity, determined using generalized linear models.

Figure 4. Not-treated (NT) areas (top) and LM-treated blocks (middle, with example close-up image of a community sample in bottom panel) in S1 and S2 recipient seres.

Figure 5. Not-treated (NT) areas (top) and LM-treated blocks (middle, with example close-up image of a community sample in bottom panel) in S2 and S3 recipient seres.

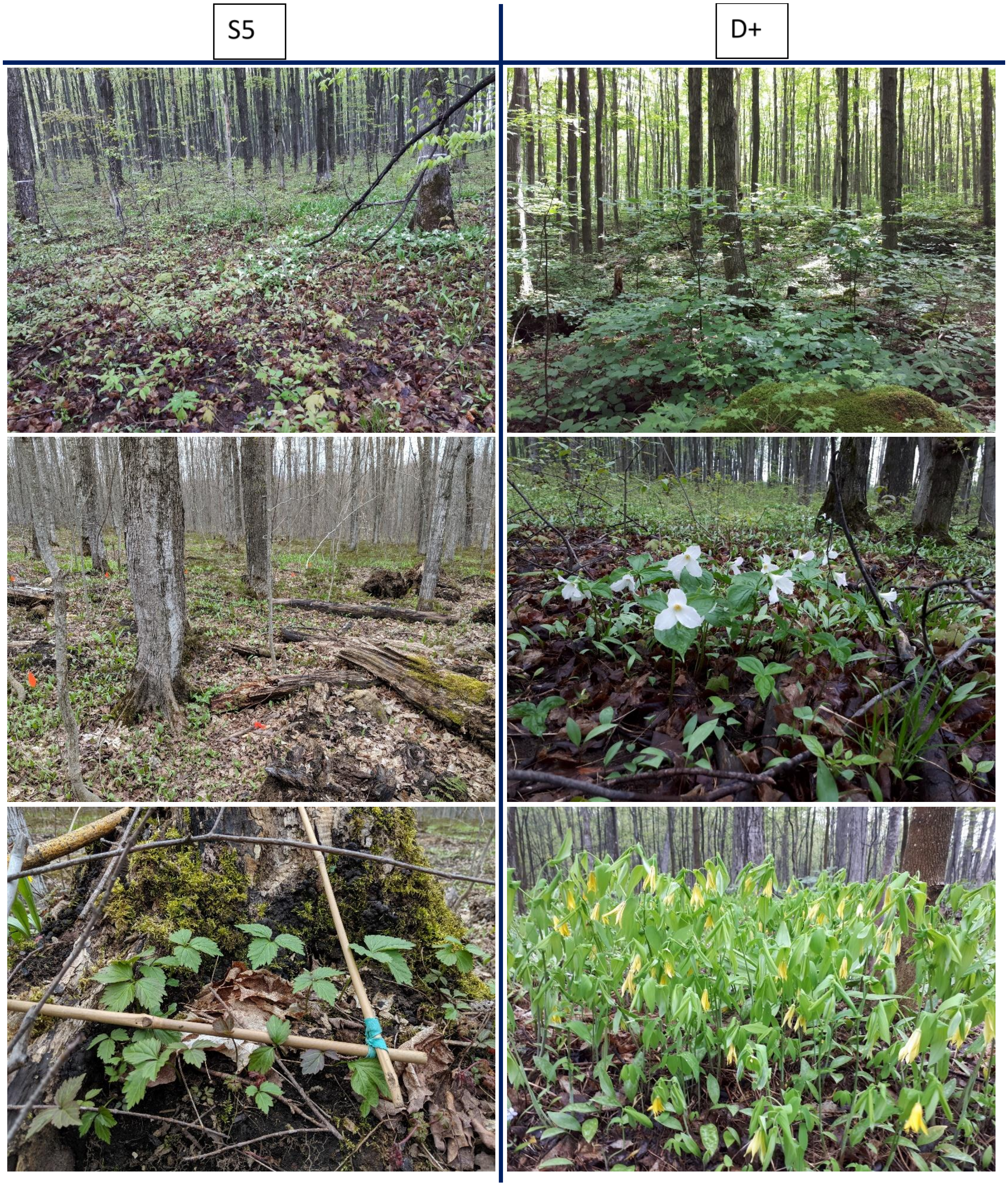

Figure 6. Not-treated (NT) areas (top) and LM-treated blocks (middle, with example close-up image of a community sample in bottom panel) in S5; images of D+, for comparison (not treated).

4.4.4 Effects of planting shrub clusters (SC) and constructing shade shelters (SS)

Efforts to establish clusters of dogwood shrubs in quadrants of LM-blocks had no apparent influence on the composition of the ground-layer plant communities – at lest not as of spring 2019. Vegetation in +LM+SC areas consistently expressed *Jsim* values within the range of variation observed in +LM areas. The construction of the shade shelters had a much more noticeable effect in the field. This is somewhat reflected by the *Jsim* values, but more so in other analyses not presented here. More time is likely needed for community responses to the shade shelters to be fully expressed. In S1, mean *Jsim* increased from 55% to 62% of the high-similarity target when comparing +LM+WD to +LM+WD+SS areas, but this difference is not statistically significant. In S2, the slightly larger increase, from 40% to 53% of the target value for the same comparison, is statistically significant. The shade shelters were not installed at the other seres due to their closed canopies, but the evidence so far suggests a moderate positive influence of the structures on the establishment of typical D+ communities when applied to LM-blocks at open sites.

4.5 Understanding how and why treatments influenced compositional similarity to D+

While *Jsim* values usefully summarize information about multiple community elements with a single number, potentially important information about the mechanisms of observed effects are unavoidably lost with this approach. Treatments or sere conditions which produced relatively low Jsim values, for example, may have failed to support establishment of “target” species (i.e. those commonly found in D+), or they may have provided an abundance of support to “non-target” species (i.e. aggressive agricultural weeds colonizing from the surrounding environments). Likewise, treatment/sere combinations that produced high *Jsim* values may have promoted D+ species, constrained non-D+ species, or both. Finally, some opportunistic woodland species such as garlic mustard employ a life-history strategy of remaining dormant as seeds in the soil seed bank until stimulated by disturbance. Species present in the LM recipient blocks which were not commonly observed in D+ may therefore have nevertheless entered the community through the LM seed bank, becoming stimulated to germinate by the act of translocation.

4.5.1 Patterns of plant species richness illuminate, but generate hypotheses more than conclusions

We will be generating insights into the mechanisms responsible for the observed *Jsim* patterns through multivariate ordination analyses of species compositions during winter 2020, but we obtained an initial glimpse into some of the more likely possibilities by complementing our analysis of *Jsim* with a similarly-structured analysis of the total number of species observed in each of the spring 2019 sample plots. By convention, we use the term “species richness” for this measure, but we acknowledge “species density” is a more accurate. First, although mature hardwood forests such as D+ are valued for their biodiversity, this does not mean each patch of ground is packed with multi-species mixtures. Earlier seres are often crowded with more species – opportunists which struggle to occupy any suitable patch as soon as it is available –and the value placed on forest biodiversity is usually more related to *which* species are present (e.g. iconic shade-adapted native wildflowers which are unlikely to be found in other habitats). Consistent with this, the average number of plant species observed in D+ plots was 7.1 (+/- 0.2) species per 0.25 m^2 while not-treated areas of S5 supported only 5.7 species per plot. Untreated areas of S1 and S2 supported slightly higher species richness (7.3 and 8.2 species per 0.25 m^2, respectively) while species richness in S3 and S4 was less than half these value (3.5 and 3.8 species per plot, respectively). The low species richness of the older plantation seres is likely because the mature trees, shaded plantation floor and thick duff layer of shed conifer needles collectively prevent immigration or establishment by potential colonists.

4.5.2 Richness in LM-recipient blocks

Species richness in the LM recipient blocks within S5 was also relatively low (5.6 species per plot), but the LM-blocks in S1-S4 all supported high levels of species richness, particularly in +WD areas. The levels of species richness supported are surprisingly close to the simple sums of the values for corresponding NT areas at each sere plus the 6-7 species per plot observed in the D+ and S5. The respective mean richness values for +LM+WD areas in S1-S4 are 16.9, 13.9, 11.6, and 11.7 species per 0.25 m^2. While this likely oversimplifies the dynamics of the establishing communities, it suggests compositional outcomes (so far, at least) are more driven by additive mixing of the different species pools than by environmental stressors filtering many species from the realized communities. These relationships are illustrated in the graph below (Fig. 7).

4.5.3 Immigration from high-richness not-treated areas may reduce compositional similarity to D+

If species richness in LM-blocks are largely additive mixtures of LM-residents and sere residents, results from the *Jsim* (compositional similarity) analysis may be explained at least in part by the resident richness differences between the open seres and those with canopy cover. From this view, the communities in LM-blocks at S1 and S2 are respectively 43% and 59% comprised of “non-LM” species (i.e. residents of the sere) while LM-blocks in S3 and S4 are only 33% and 30% comprised of “non-LM” species. It logically follows that the compositional similarity of +LM communities in the later-stage seres should be higher to D+, as there are fewer “non-D+” species to dilute the established mixture. In addition to additive mixing to form observed communities, some potential members were undoubtedly reduced in abundance or extirpated (“filtered”) from the realized compositions due to environmental stress or interactions with neighbours (we have data supporting this, but not included here). Effects of such processes are more likely to become measurable as time proceeds so our upcoming analysis of field data from the end of the 2019 growing season should improved our understanding of these drivers. One potential implication is that the success of LM application in accelerating or bypassing stages of ecosystem development may be at least as sensitive to impacts of the species pools characterizing recipient sites as to the physical environment. Efforts to overcome physical constraints though interventions such as artificial shade shelters may therefore fail to produce the desired communities if the biotic constraints (i.e. high pressure from resident and colonizing opportunistic species, or “weeds”) not also overcome.

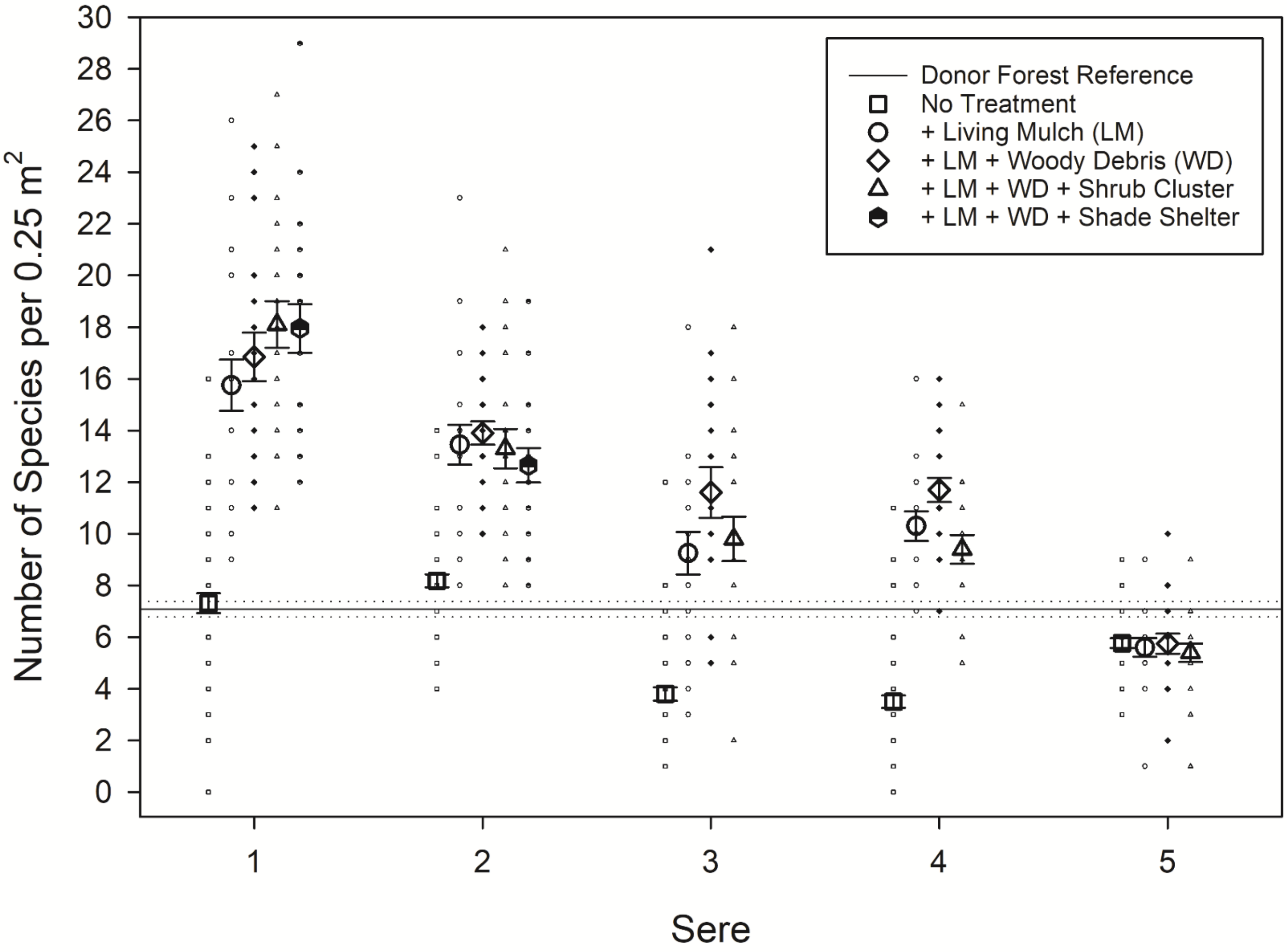

Figure 7. Responses of sample richness to recipient sere identity, and the living mulch translocation treatment levels nested within sere identity, determined using generalized linear models.

4.6 Applications for new knowledge about RERAS plant communities

Targeting LM application to locations where pressure from immigrating weed species is minimal – such as old conifer plantations -- may be the easiest way to avoid, if not overcome, the types of biotic constraints challening young afforestation or rehabilitation sites. Some buffering against the diluting effect of colonizing non-target species can likely be achieved through microhabitat interventions such as the shade shelters, especially if these are expanded to cover a larger spatial areas and sufficient time is allowed to pass. Periodic reintroductions of LM may over initial years of such an approach. While some aggressive weed species managed to establish abundantly beneath the 16 m^2 shade shelters we employed, the significant increase in *Jsim* observed in +LM+WD+SS plots compared to +LM+WD plots in S2 may be partially explained by the decrease in species richness (by 1.3 species per 0.25 m^2) for this same comparison (Fig.7). The imposed shade may therefore have increased the similarity of the underlying plant communities to D+ by preventing establishment or survival of species from surrounding NT areas. In S1, where the shade shelters did not induce a significant increase in *Jsim*, species richness increased by 1.1 species per 0.25 m^2 under the shelters rather than decreased, suggesting failure to filter non-target species caused the lack of significant impact of the shelters. Finally, the single largest difference in species richness attributable to a microhabitat treatment was the increase of 2.5 species per 0.25 m^2 associated with introduction of additional WD to LM-blocks in S3. It was at this same sere and treatment level that the highest mean *Jsim* score outside of S5 was discovered, equivalent to 86% of the target high-similarity value. This suggests the benefit of WD addition at more covered seres comes from supporting establishment of D+ species more than suppressing that of non-D+ species.

Microhabitat manipulations as well as selection of recipient seres can thus likely influence the structure of communities emerging after LM translocation both by promoting some species and by constraining others, depending on the context of application. The patterns of community response to our treatments identified so far cannot provide any final word on which ecological processes managers should attempt to manipulate for greatest success, but they do generate many useful predictions which, upon testing through continued data collection and further analysis, will help dictate refined intervention strategies to maximize desired outcomes, and minimize undesired ones, when implementing ecological enhancement programs.

5.0 INTERIM CONCLUSIONS

5.1 Goals and hypothesis of the RERAS project

The RERAS project seeks to understand the limits and opportunities which face land managers trying to achieve some of the ecological functions and values associated with mature, well-developed ecosystems such as hardwood forests, but within environments undergoing rehabilitation, afforestation, or other management aimed at offsetting or recovering from environmental degradation. More specifically, we seek to learn whether a "transplant surgery" paradigm for ecosystems may have value. We hypothesized that, in general, valued properties of ecosystems which took much time to develop may be encouraged to develop within more recently disturbed environments if the outcome of succession is treated like vital organ, not a machine which can be easily built or repaired. Principles of successful organ transplantation in animals dictate knowledge-based matching of recipients to donors; careful respect for the physiological limits of systems being operated upon; and strategic after-care based on reducing stress faced by transplants from hostile environmental factors – i.e. differences between conditions at hand versus those previously adapted to. Our hypothesis predicts that equivalents for each of these principles holds for making direct translocation of bulk "ecosystem" – an integrated whole rather than a collection of parts – from late-successional environments to earlier-stage ones successful. When successful – due to good site matching, strategic timing and placement of operations, and appropriate after-care by editing fine-scale habitat conditions to more closely approximate the donor environment – ecosystem translocation may offer unprecedented hope for effectively accelerating succession by bypassing intermediate stages, producing valued co-benefits of late-stage ecosystems including habitat for distinctive biodiversity. Our experimental work tests many specific predictions of this general hypothesis, within a system of heritage hardwood forest and surrounding former-forest, previously managed for agriculture and aggregates development but now targeted rehabilitation or afforestation initiatives.

5.2 Importance of research for aggregate sites, and of aggregate site for research

The answers to our research questions, and the potential advances to ecosystem management techniques these may bring, could have broad utility within, but also far beyond, the aggregates industry. Yet the experimental research and development required would be impossible without the participation of the aggregates industry and the existence of "living laboratories" provided by different stages of the life cycle by which aggregate resources are developed. The indispensable contribution by Walker Aggregates to the RERAS project embodies this, as the access provided to mature Niagara Escarpment forest as a donor ecosystem (prior to licensed extraction of its underlying limestone foundation) represents a potentially extremely valuable resource as "donor forest", and likely has parallels in many other natural resource development projects. Logistics and permissions required to carry out the necessary experimental manipulations at relevant scale would likely be prohibitive to researchers in most other contexts, however.

5.3 LM application is driven by conservation, recycling and restoration ethics

Ecosystem products with high ecological value arising centuries of spontaneous succession (biological legacy) must sometimes be stripped and reused in the development of natural resources. Conventional resource industry practices do not reap as much benefit from such products as possible and the ecological value goes unrealized, such as stockpiling practices which terminate living elements in soils.

Where true, changing management strategies to utilize under-valued materials through direct translocation to appropriate recipient environments should be viewed as conducive to efficient resource recycling and conservation, independent of the additional efficiencies which may accrue at rehabilitation or mitigation project where the donated material is applied. Potential benefits for managers adopting ecosystem translocation projects hinge on how well the inherent biological structures (e.g. microbial networks in soil; plant compositon) can resist and recover from stressors imposed by: i) excavation and translocation itself; ii) the new physical environment; and iii) the new suite of species interacting with the translocated organisms. The intent of our experimental design was to narrow down the field of possibilities regarding the outcome of relocating bulk quantities of mature forest topsoil, and to determine how biodiversity present in this "living mulch" (LM) responds to different qualities of: i) the potential recipient environments, such as management history and relative stage of ecosystem development; and ii) microhabitat modifications, such as creation of woody debris (WD) or shading structures (SS).

5.4 Progress made towards meeting research goals

With respect to our goals, the current progress of the RERAS research endeavour is on target. Aside from the various practical lessons learned while planning, implementing and initiating monitoring of the translocation experiment, we are now gaining valuable insight into the dominant ecological processes which will likely control the long-term success or failure of different ecosystem translocation strategies. The clearest new knowledge has resulted from careful statistical analysis of large data sets, and as the plant community surveys from spring 2019 are the only data we have analyzed deeply, our confidence about the ecological patterns is currently restricted to the time period corresponding to ca. 1.5 years post-translocation of living mulch (and one year after installation of all microhabitat modifications). Despite this unavoidable limitation, the analytical approach we developed, and our widened perspective from discovering and interpreting the patterns which have emerged so far, provide a very useful template for our next iterations of data collection and analysis.

5.5 Key findings of our interim analysis

One of the post important results from the spring 2019 vegetation data, which may be easy to overlook, is the close similarity between the D+ plant community and that which emerged in LM-treated areas of S5 (mature hardwood forest ostensibly equivalent to D+). The closeness suggests that the vegetation residing in the LM was not meaningfully impacted by the physical process of extracting, transporting and depositing the top layer of forest topsoil – likely because these steps were carried to completion in a short period of time (no stockpiling), after most vegetation had entered winter dormancy (respecting physiology of the system). As such, departures from this pattern at other recipient locations should be attributed to the new environment not the translocation process. Knowing this, the most useful lessons derived from vegetation patterns at the four earlier-stage LM recipient locations (S1-S4) are that:

i) LM application without additional alterations to microhabitat features produces a doubling to tripling of species richness at the small-plot scale (i.e. per 0.25 m^2), relative to untreated areas of recipient environments.

ii) The plant communities varied with respect to how similar their species compositions are to those residing in the donor forest (D+), but at every recipient sere, LM-treated areas shared significantly more species with D+ communities than did areas which were not treated (i.e. expressed greater values of *Jsim*, a Jaccard-based index of similarity to the D+ plant community).

iii) In most of the recipient seres, translocating additional coarse woody debris from D+ and using it to install microhabitat structures – designed to provide small-scale refuges with locally higher levels of shade, humidity, soil moisture and wood-decomposing organisms – produced a significant positive effect on the level of similarity to D+ expressed by the plant community.

iv) Planting clusters of alternate-leaved dogwood shrubs as potential agents for shading the translocated LM has yet to exhibit any meaningful effects on the ground-layer vegetation.

v) Constructing artificial shade shelters using cedar posts and horticultural shade cloth, by comparison, had a significant positive influence on the level of community similarity to D+ expressed by vegetation in affected areas of S2 (field afforested in 2015), and we saw a trend towards a similar influence in S1 (dry gravel pit undergoing spontaneous succession).

vi) If the constructed shade shelters do provide refuge to D+ biodiversity that was translocated in the LM, the full benefits of such provisioning would likely be more apparent in patterns demonstrated by the late-season rather than early-season plant communities. The mechanisms of SS effects require further investigation by likely include both protecting shade-tolerant plants from high light intensity and producing unfavourable conditions for light-favouring species which would otherwise outcompete translocated woodland species for space, nutrients or other resources. Understanding such effects better will be one goal of our upcoming analysis of the full suite of 2019 data.

5.6 Selecting seres versus altering microhabitats

Modifying microhabitat conditions at LM recipient locations may somewhat increase the power of LM to produce plant communities with high similarity to D+, these effects are dwarfed by impacts associated with the developmental status of the recipient environment. Put simply, more of the plant biodiversity present in forest LM can establish at recipient locations where a semi-closed canopy is already present – even if dominant tree types or ground coverage are dissimilar to the LM-donor – than at more open locations, such as recent afforestation or extraction sites. The nature of this effect may not be so much a case of woodland species preferring the shade, however, as it is a response to much higher immigration pressure of aggressive agricultural weeds to the more open sites. Hence, even under artificial shade mimicking the donor forest, we discovered that vegetation communities in LM-treated areas at S1 and S2 included numerous weed species typical of the surrounding fields, limiting the degree to which the plant community could compositionally resemble D+. This limitation will intensify if the woodland species are ultimately unable to coexist with the field species (which are typically superior competitors until they become limited by low light), emphasizing the need for continued monitoring and analysis of the experimental system.

The prognosis is surprisingly much more positive for the older conifer plantations, S3 and S4. Although the canopy composition, ground vegetation, litter layer, and soil chemistry are all quite distinct between conifer plantations and mature spontaneous hardwood forests – at least until plantations reach an advanced age and gradually transition into hardwood forest – translocation of LM from hardwood forest to conifer plantations consistently produced spring plant communities with very high similarity to the donor forest (i.e. 80-85% of the similarity index score produced by our adopted "ambitious target for high similarity to D+"). The plant communities which emerged from the deposited LM likely benefited from the relatively low abundance of other vegetation in the surroundings, and the fact that the biodiversity which was present tended to be less aggressively invasive than at the earlier successional sites. They also likely benefited from our necessary selection of field sites which already had moderate breaks in the canopy and openings at the ground-layer. These gaps were necessary for equipment and personnel access in the context of this experiment, but if LM application is ever to be applied at a larger scaled and mature plantations are targeted as recipients, managers would likely need to create openings to ensure moderate diversity of mixed-light environments and heterogeneous ground-layer conditions typically exploited by vegetation in old hardwood forests. Managers with access to LM resources, who seek to maximize contributions to offsetting ecological damage or otherwise combatting current ecological crises, may be wise to consider the benefits of facilitating programs for enhancing the biodiversity and ecological functioning of existing but under-performing forests, to complement ongoing forest-creation and site-rehabilitation initiatives.

5.7 Moving forward: Maintaining RERAS, advancing knowledge and maximizing its usefulness

Responses by the 2019 early-season plant communities to our experimental applications provide a valuable template for how to effectively test specific predictions made by our general "organ transplant" hypothesis, and what patterns we may expect to verify or refute as our time-series lengthens, or we analyze other ecosystem properties. Even before the 2020 growing season commences, we expect greater understanding, to be derived from analyses of the late-season 2019 data, which includes estimates of plant species cover (to date we have only analyzed species incidence patterns, not abundance) and the status of comprehensive soil properties, including: physio-chemical traits; an experimentally-determined estimate of litter decomposition rates and mechanisms; and extensive genetic information about the composition of soil bacterial, fungal and invertebrate communities.

We are enthusiastic about the potential insights into the thermodynamic states of the youngest ecosystems to receive LM application (S1-S2), which we photographed at regular intervals using a drone-mounted thermal camera. Thermal properties of recently rehabilitated or afforested sites may possibly provide rapid and inexpensive indicators of the extent to which planted or spontaneous vegetation has acclimatized to their environments, versus experiencing prohibitive stress and transitioning back to a "pre-restoration" ecosystem conformation. Our analysis of the collected thermal data combined with other studied properties offers special opportunity to test the predicted usefulness of thermal monitoring for ecosystems, in addition to providing deeper insight into the workings of our experimental system. While exhaustive analysis of all evidence lines may not be complete by spring 2020, we expect that the new information to come to light from our overwinter analyses will help refine and direct the specific plans for data collection throughout 2020. Mountains of vegetation and environmental data will undoubtedly be collected, but strategic changes from the methods employed over 2019 may be implemented if upcoming results suggest these will be beneficial.

Regardless of the fine details, we expect that one more year of monitoring the described spectrum of ecosystem responses combined with our intensifying analysis of collected data will place us in a strong empirical position to judge the short-term effectiveness and longer-term prospects for enhancing afforestation by strategically translocating and preserving biodiversity in the uppermost surface layers of old hardwood forests. Our fully-informed perspective will provide firmest foundations possible -- i.e. given constraints imposed by our current 4-year research window – for recommending both general and specific best management practices. The audience to benefit from these recommendations will include aggregate producers but also the wider afforestation, ecological restoration, and natural resource management communities. The final recommended applications and implications will take the form of a final report to TOARC, but also academic papers in both applied and theory-oriented journals, plus media in non-academic publications emphasizing resource-management frontiers and innovations.