Full title: Coupling remote sensing and eDNA to monitor environmental impact: A pilot to quantify the environmental benefits of sustainable agriculture in the Brazilian Amazon

Short title: Coupling remote sensing and eDNA to monitor environmental impact

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ABSTRACT

Monitoring is essential to ensure that environmental goals are being achieved, including those of sustainable agriculture. Growing interest in environmental monitoring provides an opportunity to improve monitoring practices. Platforms that directly monitor land cover change and biodiversity annually by coupling the wall-to-wall coverage from remote sensing and the site-specific community composition from environmental DNA (eDNA) can provide timely, relevant results for parties interested in the success of sustainable agricultural practices. To ensure that the measured impacts are due to the environmental projects and not exogenous factors, sites where projects have been implemented should be benchmarked against counterfactuals (no project) and control (natural habitat) sites. Results can then be used to calculate diverse sets of indicators customized to monitor different projects. Here, we report on our experience developing and applying one such platform to assess the impact of shaded cocoa projects implemented by the Instituto de Manejo e Certificação Florestal e Agrícola (IMAFLORA) near São Félix do Xingu, in Pará, Brazil. We used the Continuous Degradation Detection (CODED) and LandTrendr algorithms to create a remote sensing-based assessment of forest disturbance and regeneration, estimate carbon sequestration, and changes in essential habitats. We coupled these remote sensing methods with eDNA analyses using arthopod-targeted primers by collecting soil samples from intervention and counterfactual pasture field sites and a control secondary forest. We used a custom set of indicators from the pilot application of a coupled monitoring framework called TerraBio. Our results suggest that, due to IMAFLORA's shaded cocoa projects, over 400 acres were restored in the intervention area and the community composition of arthropods in shaded cocoa is closer to second-growth forests than that of pastures. In reviewing the coupled approach, we found multiple aspects worked well, and we conclude by presenting multiple lessons learned.

Key words: Agroforestry; sustainable agriculture; monitoring; remote sensing; environmental DNA; Brazil; biodiversity conservation; shaded cocoa

INTRODUCTION

Sustainable agriculture projects, which both generate income and contribute to environmental conservation, are important to address biodiversity loss, climate change, and to improve living conditions (Gomiero et al., 2011; Ruggerio, 2021; Bhagwat et al., 2008). In Brazil, sustainable agriculture approaches have been developed in response to the simultaneous pressures of combatting ongoing habitat and biodiversity loss caused by agricultural expansion and food security issues (Martinelli et al., 2010; Curtis et al., 2018; Lewis et al., 2015; Ferreira et al., 2012; Sano et al., 2010; Perfecto et al., 2007; Jezeer et al., 2017). Such practices include agroforestry, sustainability certifications, and the promotion of non-timber forest products.

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Environmental monitoring is essential to ensure that the promised impacts of sustainable agriculture are being achieved (Credit Suisse and McKinsey, 2016), and a growing interest in environmental monitoring provides an opportunity to improve monitoring practices (Threlfall et al., 2020; Hill et al., 2019, Bhagwat et al. 2008; Milder et al., 2015; Tropek et al., 2014). To improve transparency and enhance credibility, organizations need accurate, timely, and easily digestible information collected using robust methods informed by the best available science. Because the environmental impacts of projects or interventions can take many years to become evident, any monitoring system must be replicable and comparable across time. Monitoring systems should examine multiple scales to account for both individual farm and landscape-scale habitat loss or fragmentation, habitat diversity, and connectivity (Edwards et al. 2012, Ferreira et al. 2012, Tscharntke et al. 2015). Similarly, to ensure that the measured impacts are due to the project and not exogenous factors, sites where projects have been implemented should be benchmarked against counterfactuals (no projects) and control (natural habitat) sites. Indicators, or predefined metrics for assessing ecosystem services, ecosystem health and biodiversity, can be used to assess relative performance rapidly and can be designed with communication to both experts and non-experts in mind.

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Using remote sensing and environmental DNA (eDNA) approaches, systematic, broad-scale, multi-year monitoring efforts are financially and operationally feasible. Traditional approaches to monitoring forests and biodiversity, such as forest surveys and biodiversity transects, are expensive and require significant methodological and taxonomic expertise, particularly in megadiverse regions (Beng and Corlett, 2020). In contrast, products derived from remote sensing facilitate a substantial reduction in monitoring costs and simultaneously increase the timeliness of information needed to inform management (Slough et al., 2021, Townsend et al., 2021, Zhang et al., 2021). Remote sensing uses satellite and aircraft imagery and statistical approaches to detect and monitor the Earth. Existing remote sensing approaches for monitoring biodiversity allow for the evaluation of ecosystem structure and ecosystem function but are not yet extensively used in biodiversity assessment, monitoring, or conservation (Reddy et al., 2021).

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Similarly, eDNA monitoring has speed and cost advantages over traditional methods; thus, it is rapidly becoming a preferred method to monitor biodiversity, including in agricultural systems (Leese et al., 2018; Cordier et al., 2021; Ladin et al., 2021; Kestel et al., 2022). eDNA refers to genetic material obtained directly from environmental samples, such as water, soil, or air, without capturing or observing the organisms themselves (Taberlet et al., 2012), and coupled with the metabarcoding approach allows for the simultaneous identification of multiple species from a single sample (Deiner et al., 2017). eDNA sampling approaches have the ability to monitor entire taxonomic groups at multiple spatial scales depending on whether eDNA is collected from leaves, soil, animal waste, water, or air (Thomsen and Willerslev, 2015; Banerjee et al., 2022; Sales et al., 2020; Nørgaard et al., 2021; Aucone et al, 2023; Altermatt et al., 2023).

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Coupling the two technologies allows for both wall-to-wall coverage from remote sensing and the site-specific community composition from eDNA (Figure 1). Together, these methods can be used to calculate diverse sets of indicators for monitoring sustainable agriculture projects and other sustainable interventions. These indicators can include but are not limited to measures of ecosystem function such as landscape scale and site scale habitat loss and conservation, ecosystem structure, carbon sequestration through revegetation, species richness, community composition, and relative changes in community composition over time. The approaches are cost effective, and the results provide a complete picture of activities occurring across the full project site.

Here, we report on the pilot application of this approach to assess the effect of one of the sustainable agriculture initiatives of *Florestas de Valor*, created by the Instituto de Manejo e Certificação Florestal e Agrícola (IMAFLORA). *Florestas de Valor* consists of multiple initiatives including agroforestry and collection of non-timber forest products (Imaflora, 2019). We applied the coupled monitoring platform to shaded cocoa projects implemented by IMAFLORA near São Félix do Xingu, in Pará, Brazil. Shaded cocoa in Brazil is currently being supported as an alternative to unshaded cocoa and low-yield pasturelands used for cattle ranching (Johns, 1999; Gramacho et al., 1992). Key research questions included: 1) have the shaded cocoa projects contributed to conservation in the Brazilian Amazon? and 2) what impacts does shaded cocoa have on community structure and forest landscape patterns? We tested the relative effects of these management activities on biodiversity conservation and compared the results with counterfactual "business as usual" pastures and control second-growth forests to control for outside factors.

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This implementation of coupling remote sensing and eDNA for biodiversity monitoring provided an opportunity to test multiple approaches and improve the methodology. Key areas of testing included our remote sensing mapping approach, including algorithm selection, sampling design and our data collection approach, and indicator choices. These tests contributed to lessons learned that will greatly improve coupled biodiversity monitoring methods moving forward.
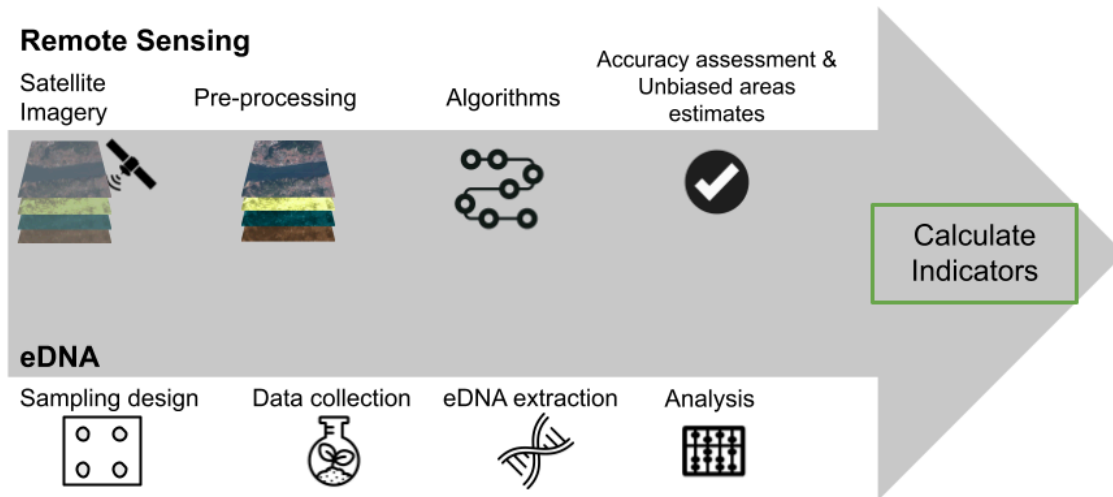
Figure 1: An overview of the proposed coupled approach to environmental monitoring. In the remote sensing component, we identify and pre-process key satellite imagery. Next, we input data into change algorithms or other models. Then, we assess the accuracy of the produced maps. In the eDNA component, we design the sampling approach by identifying locations to sample. Next, we visit sites to collect eDNA data. Then, the soil samples go through the eDNA extraction and biodiversity analysis processes. Finally, we calculate indicators from the map outputs and biodiversity results.

MATERIALS AND METHODS

STUDY AREA

The study area is located in the Xingu River basin, near the city of São Félix do Xingu, in the state of Pará, Brazil (06°38′30″South and 51°58′32″West). The region is warm moist equatorial, with dry months from May to October. The average annual rainfall is 2,041 mm. The average annual temperature is 25 °C, with minimum and maximum temperatures of 20 °C and 30 °C, respectively (INMET, 2022). Native tree species in the region include *Attalea speciosa* (Mart.) and *Cedrela odorata* L., for example (Carneiro et al., 2012). Primary forest cover was almost entirely removed from the area in the 1960s for agriculture and was slowly replaced by secondary vegetation, including forest. Subsequently, farmers cleared secondary forests for cattle grazing (Mertens et al., 2002; Schmink et al., 2019).

39. Instituto Nacional de Meteorologia (INMET). Banco de dados meteorológicos. Accessed on April 27, 2022.
40. Carneiro, V., Sablayrolles, P., Oliveira, C., & das Graças Sablayrolles, M. (2012). Composição e Diversidade Florística de Remanescentes Florestais Presentes em Lotes de

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More recently, shade-grown cacao (*Theobroma cacao* L.) has been promoted in the region as a sustainable alternative (Schroth et al., 2016; Schmink et al., 2019; Braga et al., 2019). Shaded cocoa is thought to reduce agricultural inputs, disease susceptibility, and drought susceptibility as well as increase food security and environmental benefits (Braga et al., 2019; Schroth et al., 2016). There are two phases to cultivating shade trees in cocoa agroforestry systems. First, specific shade trees, including banana and papaya, are cultivated while native regeneration occurs, and second, these cultivated trees are thinned, and native shade trees become dominant (Braga et al., 2019). During the first phase, annual crops like cassava and maize are also grown, with cassava chosen to increase the nitrogen content in the soil (Braga et al., 2019; IMAFLORA, personal communication). Native shade trees include *Apuleia leiocarpa* (Vogel) J.F. Macbr., *Bagassa guianensis* Aubl., *Pouteria macrophylla* (Lam.) Eyma, *Erythrina verna* Vell., *Pouteria pariry* (Ducke) Baehni, *Chrysophyllum cuneifolium* (Rudge) A. DC., *Perebea guianensis* Aubl., *Spondias mombin* L., *Colubrina glandulosa* Perkins, *Cenostigma tocantinum* Ducke, *Annona mucosa* Jacq., *Handroanthus serratifolius* (Vahl) S.Grose, *Inga edulis* Mart. and *Samanea tubulosa* (Benth.) Barneby and J. W. Grimes (Braga et al., 2019).

43. Schroth G, Garcia E, Griscom BW, Teixeira WG, Barros LP (2016) Commodity production as restoration driver in the Brazilian Amazon? Pasture re-agro-forestation with cocoa (Theobroma cacao) in southern Para. Sustain Sci 11:277–293

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IMAFLORA is a nonprofit partner in the SERVIR Amazonia consortium based in Brazil. IMAFLORA maintains a database of 150 farms participating in multiple agricultural practices and has worked with a subset of these farmers to implement shaded cocoa practices over the past 20 years (Neves et al., 2015; Figure 2). Within this context, our study boundary encapsulated the farms partnering with IMAFLORA.

45. Neves, Andressa, Nachtergaele, Marcos Froes, and Gonçalves, Eduardo Trevisan. (2015). São Félix do Xingu: farmers lead exchange of experiences in cocoa management

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https://www.imaflora.org/noticia/sao-felix-do-xingu-agricultores-lidera. Accessed
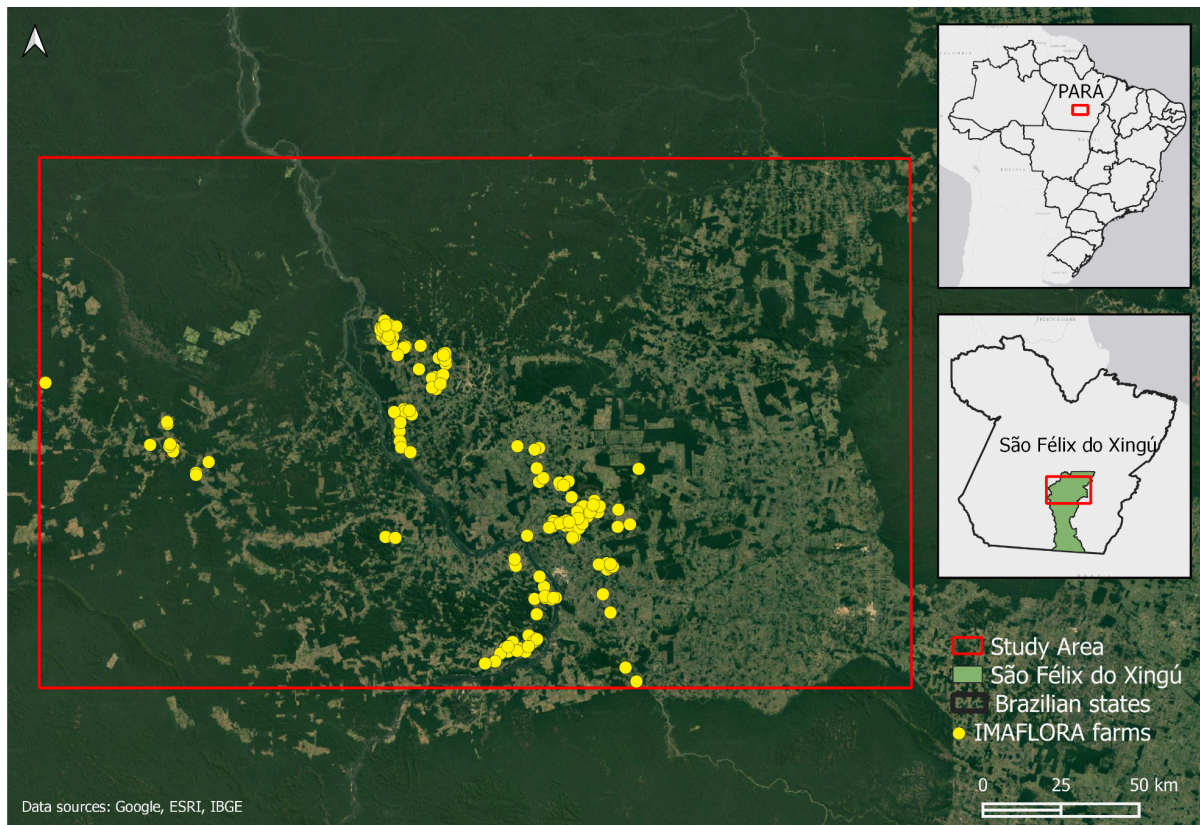9/7/2022.

Figure 2: Study area in the state of Pará, Brazil. Yellow dots represent the farms that have partnered with IMAFLORA.

REMOTE SENSING METHODS

DISTURBANCE MAPPING

Forest disturbances were mapped using a combination of two pixel-based methods: the Continuous Degradation Detection (CODED) algorithm (Bullock et al., 2020a; Bullock et al., 2020b), and the Landsat-based detection of Trends in Disturbance and Recovery (LandTrendr) algorithm (Kennedy et al., 2018). Both algorithms utilize Landsat collections in a time series approach (30 m spatial resolution). Here, disturbances are separated into deforestation and forest degradation. We consider deforestation a permanent conversion of forested land to non-forested land (FAO, 2007), and degradation a process that does not lead to a categorical land cover change but shows the loss in tree cover canopy (Aryal et al., 2021; Souza et al., 2005). The changes were mapped for the 5-year period 2010-2015, when most of the interventions occurred. The results of the two algorithms were combined and evaluated for accuracy.

46. Bullock, E. L., Nolte, C., Reboredo Segovia, A. L., & Woodcock, C. E. (2020a). Ongoing forest disturbance in Guatemala's protected areas. Remote sensing in ecology and conservation, 6(2), 141-152.
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50. Aryal, R. R., Wespestad, C., Kennedy, R., Dilger, J., Dyson, K., Bullock, E., ... & Tenneson, K. (2021). Lessons Learned While Implementing a Time-Series Approach to Forest Canopy Disturbance Detection in Nepal. Remote Sensing, 13(14), 2666.
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CODED is a freely available tool on Google Earth Engine (GEE), an online planetary-scale computing platform for remote sensing and satellite imagery analysis (Gorelick et al., 2017). CODED uses all the Landsat imagery available from Landsat collections 5, 7, and 8 to perform a subpixel spectral mixture analysis (SMA), analyzing time series changes in the Normalized Degradation Fraction Index (NDFI) (30 m resolution; Souza et al., 2005, Bullock et al., 2020a). The spectral index of choice was the NDFI to be in accordance with CODED and also since previous studies have shown that NDFI is more sensitive to disturbances in tropical forests compared to the commonly used Normalized Difference Vegetation Index (NDVI), which tends to show higher variability (Schultz et al., 2016, Bullock et al., 2020a; Bullock et al., 2020b). We used 3,000 training points labeled as forest or non-forest and tested chi-squared values of both 0.9 and 0.99, which controls the width of the change-detection moving time window (Aryal et al., 2021). We defined the required number of sequential out-of-range NDFI values to flag an event as four times. In post-processing, we required the magnitude of these flagged disturbances to be above 0.4 in order to be considered severe enough to include in the final map. In this manner CODED is able to detect low-severity disturbances, which is often characteristic of the more difficult-to-detect forest degradation events, as opposed to forest loss. These parameter values were suggested for work in the Amazon by CODED's designer (Eric Bullock, personal communication, July 22, 2021). The final CODED output is a map with 30 m pixels labeled as non-forest, stable forest, deforestation, or degradation.

52. Gorelick, N., Hancher, M., Dixon, M., Ilyushchenko, S., Thau, D., and Moore, R. (2017). Google Earth Engine: Planetary-scale geospatial analysis for everyone. *Remote sensing of Environment*, *202*, 18-27.

53. Schultz, Michael, et al. "Performance of vegetation indices from Landsat time series in deforestation monitoring." International journal of applied earth observation and geoinformation 52 (2016): 318-327. https://doi.org/10.1016/j.jag.2016.06.020

Next, we used the LandTrendr algorithm, also freely available and hosted on GEE. LandTrendr is a collection of algorithms used to detect land cover change through time series analysis of Landsat imagery. Specifically, LandTrendr constructs an image collection by creating medoid composites of Landsat 5, 7, and 8 images, resulting in one image per year. The medoid image compositing approach compares each pixel's spectral band values to the median spectral values of those bands across all images within the date-constrained collection for a given year. The pixel with spectral values closest to the median value, determined by Euclidean spectral distance, is then selected (Kennedy et al., 2018). This tool aims to filter out inter-annual noise in spectral signals and generate trajectory-based time series estimates and accomplishes this through simplifying multi-year spectral trajectories into several straight-line segments that capture the progressing changes of the signal (Kennedy et al., 2018). The algorithm was parameterized to estimate the "greatest" disturbance, and specific parameters are mostly in accordance with Reygadas et al. (2021) and Fragal et al. (2016). We further classified the disturbance events from LandTrend as degradation and deforestation using MapBiomas classification in 2015 (Souza et al., 2020). Disturbances classified as forest by MapBiomas in 2015 were considered degradation, whereas disturbances classified as non-forest were classified as deforestation.

54. Reygadas, Y., Spera, S., Galati, V., Salisbury, D. S., Silva, S., & Novoa, S. (2021). Mapping forest disturbances across the Southwestern Amazon: tradeoffs between open-source, Landsat-based algorithms. Environmental Research Communications, 3(9), 091001.
55. Fragal, Everton Hafemann, Thiago Sanna Freire Silva, and Evlyn Márcia Leão de Moraes Novo. "Reconstructing historical forest cover change in the Lower Amazon floodplains using the LandTrendr algorithm." Acta Amazonica 46 (2016): 13-24.
56. Souza Jr, C. M., Z. Shimbo, J., Rosa, M. R., Parente, L. L., A. Alencar, A., Rudorff, B. F., ... & Azevedo, T. (2020). Reconstructing three decades of land use and land cover changes in brazilian biomes with landsat archive and earth engine. Remote Sensing, 12(17), 2735. https://doi.org/10.3390/rs12172735

Lastly, the final disturbance map was generated by overlapping the CODED and LandTrendr maps and using a rule-based assignment, where pixels with classification disagreements were reclassified as: degradation if at least one of the outputs was classified as degradation, deforestation if none of the outputs were classified as degradation and at least one classified as deforestation, and, consequently, forest if both outputs were classified as forest. We used this system because degradation was underestimated in previous studies (Souza et al., 2013; Aryal et al., 2021; Reygadas et al., 2021).

57. Souza, J.; Siqueira, J.V.; Sales, M.H.; Fonseca, A.V.; Ribeiro, J.G.; Numata, I.; Cochrane, M.A.; Barber, C.P.; Roberts, D.A.; Barlow, J. Ten-Year Landsat classification of deforestation and forest degradation in the Brazilian Amazon. Remote Sens. 2013, 5, 5493–5513. https://doi.org/10.3390/rs5115493

REGENERATION MAPPING

Similarly to the disturbance mapping, we used LandTrendr to map forest regeneration following previous research (Fragal et al., 2016; Hua et al., 2021). LandTrendr was set up to detect upward trends in the spectral signature of forested areas. The same NDFI index was used, and the parameterization was set to estimate the "greatest" gain and specific parameters adjusted as an attempt to capture short-time regeneration given the 5-year period.

58. Hua, Jianwen, et al. "Improved Mapping of Long-Term Forest Disturbance and Recovery Dynamics in the Subtropical China Using All Available Landsat Time-Series Imagery on Google Earth Engine Platform." IEEE Journal of Selected Topics in Applied Earth Observations and Remote Sensing 14 (2021): 2754-2768.

Silva Junior et al. (2020) developed maps of annual secondary forest extent, age, increment, and loss within Brazil for 1986-2019 based on maps by the Brazilian Annual Land Use and Land Cover Mapping Project (MapBiomas) (Souza et al., 2020). Using MapBiomas data, Silva Junior et al. (2020) determined that secondary forest growth is present when a pixel with an anthropic cover classification (e.g., pasture or agriculture) becomes a forest cover pixel (excluding mangroves and forest plantations) the following year. We utilized the same methodology to generate a map of secondary forests, here also considered as regeneration, from 2010 to 2015. These maps were also Landsat-based with a 30 m spatial resolution.

59. Silva Junior, C. H. L., Heinrich, V. H., Freire, A. T., Broggio, I. S., Rosan, T. M., Doblas, J., ... and Aragão, L. E. (2020). Benchmark maps of 33 years of secondary forest age for Brazil. Scientific data, 7(1), 1-9.
60. Souza, C. M., Z Shimbo, J., Rosa, M. R., Parente, L. L., A Alencar, A., Rudorff, B. F., ... & Azevedo, T. (2020). Reconstructing three decades of land use and land cover changes in brazilian biomes with landsat archive and earth engine. Remote Sensing, 12(17), 2735.

The final regeneration map was a combination of the LandTrendr output with the output from Silva Junior et al. (2020) methodology where a pixel is mapped as "regeneration" if at least one of the outputs was mapped as "regeneration", in an "all-inclusive" approach. This way, we could assess this approach's accuracy and inform future TerraBio work.

ACCURACY ASSESSMENT OF MAPPING PRODUCTS

An independent validation effort was conducted leveraging high- and medium-resolution optical imagery (Planet NICFI mosaics, Google Earth Pro basemaps, Sentinel-2, and Landsat

Collections) and ancillary datasets (MapBiomas products, the Global Forest Canopy Height 2019, and NDFI time series) to assess classes on the ground (Planet, 2021; Buchhorn et al., 2020; ESA, 2015; Farr et al., 2007; Potapov et al., 2021; Olofsson et al., 2014). Visual interpretation was done in Collect Earth Online (CEO), a free and open-source web-based tool that facilitates data collection and validation (Bey et al., 2016; Saah et al., 2019). The interpreter utilized a decision tree approach for classifying the validation samples.

61. Planet. (2021) Planet and NICFI partnership data
62. Buchhorn, M., Lesiv, M., Tsendbazar, N. E., Herold, M., Bertels, L., and Smets, B. (2020). Copernicus global land cover layers—collection 2. Remote Sensing, 12(6), 1044.
63. ESA, 2015. SENTINEL-2 User Handbook, vol. 1, pp. 64.
64. Farr, T. G. et al., 2007, The Shuttle Radar Topography Mission, Rev. Geophys., 45, RG2004, doi:10.1029/2005RG000183
65. Potapov, P., Li, X., Hernandez-Serna, A., Tyukavina, A., Hansen, M. C., Kommareddy, A., ... and Hofton, M. (2021). Mapping global forest canopy height through integration of GEDI and Landsat data. Remote Sensing of Environment, 253, 112165.
66. Olofsson, Pontus, et al. "Good practices for estimating area and assessing accuracy of land change." Remote Sensing of Environment 148 (2014): 42-57. https://doi.org/10.1016/j.rse.2014.02.015.
67. Bey, A., Sánchez-Paus Díaz, A., Maniatis, D., Marchi, G., Mollicone, D., Ricci, S., ... and Miceli, G. (2016). Collect earth: Land use and land cover assessment through augmented visual interpretation. Remote Sensing, 8(10), 807.
68. Saah, D., Johnson, G., Ashmall, B., Tondapu, G., Tenneson, K., Patterson, M., ... and Chishtie, F. (2019). Collect Earth: An online tool for systematic reference data collection in land cover and use applications. Environmental Modelling and Software, 118, 166-171.

For the validation of the disturbance and regeneration maps, the sample points were 30 by 30 meters square, mimicking the map output pixel sizes. The validation of both change maps (disturbance and regeneration) was done using the same set of points. We also used a simple semi-random sampling design with proportional class distribution where we extracted 600 points from both maps, therefore, 100 points for each class: degradation, stable non-forest, stable forest, deforestation, regeneration, different events (Table S1). A "different events" category was used to capture locations where the mapping methods determined a different class for the same location. This class can include regeneration events on non-forest areas followed by degradation or deforestation or disturbance events, most likely deforestation, followed by regeneration. The accuracy metrics (overall, user, and producer accuracies) and unbiased area estimates for each class were calculated through the ratio estimator approach (Stehman, 2014) for when the strata are different from the map classes since we used the same sample points for disturbance map and regeneration map.

69. Stehman, S. V. (2014). Estimating area and map accuracy for stratified random sampling when the strata are different from the map classes. International Journal of Remote Sensing, 35(13), 4923-4939.

eDNA METHODS

eDNA FIELD DATA COLLECTION

We defined our sampling frame as the 150 farms partnering with IMAFLORA. Within this sampling frame, we verified which farms included our project sites, or farms with mature shaded cocoa, by checking average vegetation height of cocoa fields (minimum average canopy height of 7.5m; Potapov et al., 2021) and verifying that fields contained greater than 25% canopy cover in CEO. We identified 49 farms containing mature shaded cocoa.

Our sampling design was chosen to reduce the influence of exogenous variables that influence biodiversity in order to maximize our statistical power in detecting differences between project and counterfactual sites. Key exogenous variables include the amount and configuration of forest both within the farm boundaries and in a 250m buffer (Gustafson, 1998; Jordan et al., 1995; Turner et al., 2001; Brudvig, 2011; de Souza Leite et al., 2013). To represent these exogenous influences, we selected a suite of landscape ecology metrics that best captured landscape variance using principal component analysis (PCA; McCabe 1984; Pacheco et al., 2013). Metrics used for clustering included within the farm boundaries: total forest area, number of forest patches, forest percentage of farm, forest contiguity, and forest aggregation index; and within the 250m buffer included: total forest area, forest percentage of landscape, and forest aggregation index (Hesselbarth et al., 2019). We used these landscape metrics to assign each farm to a cluster using Ward's hierarchical clustering with the Euclidean distance matrix (Maechler et al., 2019).

70. Gustafson, E. J. (1998). Quantifying landscape spatial pattern: what is the state of the art? Ecosystems, 1(2), 143-156.
71. Jordan, G., Baskent, E. Z., & Whittaker, G. A. (1995). Spatial forest modelling and landscape management.
72. Turner, M. G., Gardner, R. H., O'neill, R. V., & O'Neill, R. V. (2001). Landscape ecology in theory and practice (Vol. 401). Springer New York.
73. Brudvig, L. A. (2011). The restoration of biodiversity: where has research been and where does it need to go? American journal of botany, 98(3), 549-558.
74. de Souza Leite, M., Tambosi, L. R., Romitelli, I., & Metzger, J. P. (2013). Landscape ecology perspective in restoration projects for biodiversity conservation: a review. Natureza & Conservação, 11, 108-118.
75. McCabe, G. P. (1984). Principal variables. Technometrics, 26(2), 137-144.
76. Pacheco, J., Casado, S., & Porras, S. (2013). Exact methods for variable selection in principal component analysis: Guide functions and pre-selection. Computational Statistics & Data Analysis, 57(1), 95-111.

77. Hesselbarth, M.H.K., Sciaini, M., With, K.A., Wiegand, K., Nowosad, J. 2019. landscapemetrics: an open-source R tool to calculate landscape metrics. - Ecography 42:1648-1657(ver. 0).
78. Maechler, M., Rousseeuw, P., Struyf, A., Hubert, M., Hornik, K. (2019). cluster: Cluster Analysis Basics and Extensions. R package version 2.1.0.

Following cluster assignment for each farm, we used stratified random sampling to select five project sites and five counterfactual sites with the same proportion of each cluster in each group of sites. We also used the same process to select two back-up sites for each group. We avoided excluding clusters since that would have changed the sampling frame. In addition, local experts identified five forest sites that represented the oldest known second growth forest areas in the study area (IMAFLORA, personal communication). However, the field team experienced difficulties accessing sites, weather delays, and COVID delays. This led to only one second growth forest being sampled, reduced numbers of plots sampled in one shaded cocoa field and two pastures, and the use of both shaded cocoa backup sites and one pasture backup site.

A total of 38 plots were sampled, with 18 plots from 5 shaded-cocoa sites, 17 from 5 pasture sites and 3 from one forest site. Within each site, we randomly placed either 3 or 4 50m x 50m plots depending on the field size, with at least 25m between plots. Each plot had four sub-samples, set back from the edge of the plot by 12.5m, to better sample the heterogeneity found within the plot. Approximately 30g of the topsoil (between 0-5 cm deep) was collected for each sub-sample, making sure to avoid non-soil matter including leaf litter, and sub-samples were pooled to represent one sample per plot. To avoid sample contamination and ensure consistency across samples, a protocol was followed during sampling that included the use of disposable sampling materials and gloves and samples were labeled and individually packed according to plots and sites to ensure no cross-contamination occurred (Foucher et al., 2020; detail of methods see S2 File). Following collection, soil samples were stored with silica desiccant bags (minimum of 10g per sample) protected from heat and sunlight to prevent DNA degradation and later transported to the laboratory facilities for further laboratory analysis.

79. Foucher, A., Evrard, O., Ficetola, G. F., Gielly, L., Poulain, J., Giguet-Covex, C., … Poulenard, J. (2020). Persistence of environmental DNA in cultivated soils: implication of this memory effect for reconstructing the dynamics of land use and cover changes. Scientific Reports, 10(1), 1–12. https://doi.org/10.1038/s41598-020-67452-1

eDNA EXTRACTION

Soil samples were pre-processed in a Department for Environment, Food & Rural Affairs (DEFRA) licensed laboratory facility. To avoid contamination, samples were handled in a pre-PCR laboratory, using disposable tools and gloves, following standard decontamination procedures (i.e., use of bleach to clean surfaces and equipment), and personnel wore disposable full-body suits when handling the samples.

The extraction method was conducted using 2 g of mixed soil (per analyzed plot) and following the Mu-DNA soil DNA extraction protocol described by Sellers et al. (2018). Negative controls were included, comprising DNA extraction blanks containing only the required buffers.

80. Sellers, G. S., Di Muri, C., Gómez, A., and Hänfling, B. (2018). Mu-DNA: a modular universal DNA extraction method adaptable for a wide range of sample types. Metabarcoding and Metagenomics, 2, e24556.

Following DNA extractions, DNA amplification was conducted using three sets of primers targeting two partial mitochondrial genes. First, vertebrate specific primers were used targeting ~106 bp of the 12S rRNA gene (Riaz et al., 2011; forward primer 5'-TAGAACAGGCTCCTCTAG-3' and reverse primer 5'-TTAGATACCCCACTATGC-3'). Second, to detect arthropods, DNA extracts were amplified using two primer sets targeting different short inserts of the mtDNA COI gene. The Zeale primer set (Zeale et al. 2011) was used to amplify a ~157 bp fragment, and the Gillet primers (Gillet et al., 2015; Hebert et al. 2003; Hajibabaei et al. 2011) were used to amplify a ~133 bp section (Zeale: forward primer 5'-AGATATTGGAACWTTATATTTTATTTTTGG-3' and reverse primer 5'-WACTAATCAATTWCCAAATCCTCC-3'; Gillet: forward primer 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGNNNNNNNNATTCHACDAAYCAYAA RGAYATYGG-3' and reverse primer 5'-CCTCTCTATGGGCAGTCGGTGATNNNNNNNNACTATAAAARAAAATYTDAYAAA DGCRTG-3').

81. Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P. & Coissac, E. (2011) ecoPrimers: inference of new DNA barcode markers from whole genome sequence analysis. Nucleic Acids Research, 39, e145.
82. Zeale, M. R., Butlin, R. K., Barker, G. L., Lees, D. C., & Jones, G. (2011). Taxon‑specific PCR for DNA barcoding arthropod prey in bat faeces. Molecular ecology resources, 11(2), 236-244.
83. Gillet, F., Tiouchichine, M. L., Galan, M., Blan, F., Némoz, M., Aulagnier, S., & Michaux, J. R. (2015). A new method to identify the endangered Pyrenean desman (Galemys pyrenaicus) and to study its diet, using next generation sequencing from faeces. Mammalian Biology, 80(6), 505-509.
84. Hebert, P. D., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270(1512), 313-321.
85. Hajibabaei, M., Shokralla, S., Zhou, X., Singer, G. A., & Baird, D. J. (2011). Environmental barcoding: a next-generation sequencing approach for biomonitoring applications using river benthos. PLoS one, 6(4), e17497.

PCR reactions consisted of 12.5 µl Master Mix, 7.5 µl molecular grade water, 2 µl of DNA template and 1 µl of the forward and reverse of each primer. The PCR conditions for the Riaz

primer followed the Kelly et al. (2014) methodology, consisting of an incubation of 5 minutes at 95 °C, then 35 cycles for 15 seconds at 95 °C, 30 seconds at 57 °C, ending with 30 seconds at 72°C. PCR conditions of the Gillet and Zeale primers followed the protocols set by Browett et al. (2021). Gillet cycles included an initial 15-minute denaturation at 95 °C, then 10 cycles for 30 seconds at 94 °C, 45 seconds at 49 °C, 30 seconds at 72 °C, 30 cycles of 30 seconds at 95 °C, 45 seconds at 47 °C and 30 seconds at 72 °C, with a final extension following of 10 minutes at 72 °C. Zeale PCR conditions began with a 15-minute denaturation at 95 °C, then 40 cycles of 20 seconds each at 95 °C, 30 seconds at 55 °C and 1 minute at 72 °C, with a final extension of 7 minutes at 72 °C. PCR cycles were authenticated by electrophoresis in a 1.2% agarose gel stained with GelRed. PCRs were run in triplicates, and the success of the reactions was determined by electrophoresis on a 1.5% agarose gel. Four PCR blanks were included in each library to account for putative contaminations arising in the amplification steps. In total, 44 samples were analyzed per library, 38 eDNA soil samples, two extraction blanks and four PCR blanks. A left-sided size selection was performed using 1.2× Agencourt AMPure XP (Beckman Coulter) and the KAPA HyperPrep kit (Roche) was used to construct the Illumina libraries using the dual-indexed adapters. Libraries were quantified using the NEBNext qPCR quantification kit (New England Biolabs) and pooled in equimolar concentrations. Two Illumina MiSeq sequencing runs were conducted, one MiSeq v2 Reagent Kit (2 × 150 bp paired-end reads) and one MiSeq v3 Reagent Kit (2 × 300 bp paired-end reads).

86. Kelly RP, Port JA, Yamahara KM, Crowder LB. Using environmental DNA to census marine fishes in a large mesocosm. PloS one. 2014 Jan 15;9(1):e86175.
87. Browett, S.S., Curran, T.G., O'Meara, D.B. et al. Primer biases in the molecular assessment of diet in multiple insectivorous mammals. Mamm Biol 101, 293–304 (2021). https://doi.org/10.1007/s42991-021-00115-4

Bioinformatic steps were conducted as described in Browett et al. (2021). In brief, bioinformatic analysis used the OBITools 1.2.2 metabarcoding package (Boyer et al., 2016). Read quality was assessed using FastQC, Illumina pairedend aligned paired-end reads, and ngsfilter demultiplexed samples and removed primers. The obigrep command performed size selection by eliminating artifacts and ambiguous reads. Vsearch (Rognes et al., 2016) clustered unique sequences and removed chimeras using uchime-denovo (Edgar et al., 2011). Sumaclust clustered sequences into Molecular Operational Taxonomic Units (MOTUs) at thresholds of 0.95-0.98. Taxonomic assignment relied on Basic Local Alignment Search Tool (BLAST, specifically blastn) against Genbank, with a minimum of 90% alignment and >80% similarity (Benson et al., 2013). Species-level assignment required ≥98% identity, while MOTUs at 95%-98% or with multiple species were assigned at the genus level. MOTUs between 93%-95% were assigned to the family level, and MOTUs between 90%-93% were assigned to the order level (Browett et al. 2021). Sequences were retained when they could be identified at least to the Class level. A final filtering step was conducted, including the removal of putative contaminants, tag-jumping (MOTUs represented by less than 0.01% of the total reads were removed from each sample), and non-target taxa (e.g., Human DNA). Additionally, molecular operational taxonomic units (MOTUs) were retained when the total number of reads was over 50 (Valsecchi et al., 2020).

MOTUs were considered compositional data and treated as such (Gloor et al., 2017; Calle, 2019; McKnight et al., 2018), except for when indicators called for species abundance measures where reads were used (Di Muri et al., 2020).

88. Boyer F, Mercier C, Bonin A, Le Bras Y, Taberlet P, Coissac E. obitools: A unix‑inspired software package for DNA metabarcoding. Molecular ecology resources. 2016 Jan;16(1):176-82.
89. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool for metagenomics. PeerJ. 2016 Oct 18;4:e2584.
90. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 2011 Aug 15;27(16):2194-200.
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92. Valsecchi, E, Bylemans, J, Goodman, SJ orcid.org/0000-0003-4118-8575 et al. (5 more authors) (2020) Novel universal primers for metabarcoding environmental DNA surveys of marine mammals and other marine vertebrates. Environmental DNA, 2 (4). pp. 460-476. ISSN 2637-4943
93. Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., & Egozcue, J. J. (2017). Microbiome datasets are compositional: and this is not optional. Frontiers in microbiology, 8, 2224.
94. Calle, M. L. (2019). Statistical analysis of metagenomics data. Genomics & informatics, 17(1).
95. McKnight, D. T., Huerlimann, R., Bower, D. S., Schwarzkopf, L., Alford, R. A., & Zenger, K. R. (2019). Methods for normalizing microbiome data: an ecological perspective. Methods in Ecology and Evolution, 10(3), 389-400.
96. Di Muri, C., Lawson Handley, L., Bean, C. W., Li, J., Peirson, G., Sellers, G. S., ... & Hänfling, B. (2020). Read counts from environmental DNA (eDNA) metabarcoding reflect fish abundance and biomass in drained ponds. Metabarcoding and Metagenomics, 4, 97-112.

eDNA ANALYSIS

The four initial biodiversity indicators and four proposed indicators were calculated based on the resulting data. For indicators using key species, we defined key species for mammals as threatened native species and also excluded domesticated and invasive species from analysis more broadly (IUCN, 2021). We defined key species for arthropods as members of the Hymenoptera and Lepidoptera orders as important pollinator species, including for coffee crops (Latini et al., 2020; Vaissière et al., 2008). These indicators require BLAST to match sequences to the Order level. As has been previously reported in other studies (e.g., Browett et al. 2021), the Gillet primers were notably better at detecting members of Hymenoptera, and the Zeale primers were better at detecting members of Lepidoptera. Due to the limited overlap between the

two primer sets, we combined the resulting datasets for further analysis. For determining if any MOTUs were associated with (indicative of) either cocoa fields or pasture and the ecological conditions found there (Bakker, 2008; De Caceres et al., 2012) we used the multipatt function {indicspecies} with a custom wrapper (De Caceres and Legendre, 2009; Dyson, 2023; Dyson, 2020).

97. IUCN. 2021. The IUCN Red List of Threatened Species. Version 2021-3. https://www.iucnredlist.org. Accessed on 29 April 2022.

98. Latini, A. O., Silva, D. P., Souza, F. M. L., Ferreira, M. C., de Moura, M. S., and Suarez, N. F. (2020). Reconciling coffee productivity and natural vegetation conservation in an agroecosystem landscape in Brazil. Journal for Nature Conservation, 57, 125902.

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100. Bakker, J. D. (2008). Increasing the utility of indicator species analysis. Journal of Applied Ecology, 45(6), 1829-1835.

101. De Cáceres, M., Legendre, P., Wiser, S. K., and Brotons, L. (2012). Using species combinations in indicator value analyses. Methods in Ecology and Evolution, 3(6), 973-982.

102. De Caceres, M., Legendre, P. (2009). Associations between species and groups of sites: indices and statistical inference. Ecology, URL http://sites.google.com/site/miqueldecaceres/

103. Dyson K. (2023). Custom Community Ecology Helper R Scripts. <https://github.com/kdyson/R_Scripts> accessed 6 June 2023. DOI: 10.5281/zenodo.8011929

104. Dyson, K. (2020). Conserving native trees increases native bird diversity and community composition on commercial office developments. Journal of Urban Ecology, 6(1), juaa033.

For community-based indicators, we used all sequences identified by BLAST as Arthropods, requiring identification to the Phylum level. We first accounted for zeros in the dataset using zCompositions (cmultRepl; Palarea-Albaladejo and Martín-Fernández, 2015), then transformed the data using compositions (cdt.acomp; Van den Boogaart and Tolosana-Delgado, 2008). We calculated Aitchison distance between sites and between treatments using the Euclidean distance matrix (Ladin et al., 2021; Gloor et al., 2016; Aitchison, 1983). We created PCA plots using the transformed compositional data (Gloor et al., 2016; Aitchison, 1983).

105. Palarea-Albaladejo, J., and Martín-Fernández, J. A. (2015). zCompositions—R package for multivariate imputation of left-censored data under a compositional approach. Chemometrics and Intelligent Laboratory Systems, 143, 85-96.

106.   Van den Boogaart, K. G., and Tolosana-Delgado, R. (2008). "Compositions": a unified R package to analyze compositional data. Computers and Geosciences, 34(4), 320-338.

107.   Gloor G. B., Wu J. R., Pawlowsky-Glahn V., Egozcue J. J. (2016). It's all relative: analyzing microbiome data as compositions. Ann. Epidemiol. 26, 322–329. 10.1016/j.annepidem.2016.03.003

108.   Aitchison J. (1983). Principal component analysis of compositional data. Biometrika 70, 57–65. 10.1093/biomet/70.1.57

Where needed, we used linear mixed models to account for the repeated sampling design to test the difference between projects and counterfactuals (multiple plots per field; lmer {lme4}; Bates et al., 2015). Significance tests were performed using Anova and Type II Sums of Squares ({car}; Fox and Weisberg, 2019). All data analysis was conducted in R (R Core Team, 2022) and all code is available on GitHub (https://github.com/sig-gis/TerraBioPilot) and will be published in a public repository.

109.   Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software. 2015 Oct 7;67:1-48.

110.   Fox, J. and Weisberg, S. (2019). An {R} Companion to Applied Regression, Third Edition. Thousand Oaks CA: Sage. URL: https://socialsciences.mcmaster.ca/jfox/Books/Companion/

111.   R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

CARBON CALCULATIONS

Carbon sequestration through revegetation estimates was calculated using modified methods based on the New IPCC Tier-1 Global Biomass Carbon Map for the Year 2000 (Gibbs and Ruesch, 2008). Tier 1 carbon estimates are defined using a look-up table which has an associated carbon value based on Global Land Cover 2000 (GLC2000; Bartholome and Belward, 2005) cover type, ecofloristic zone, continental region, and frontier forest designation. We assumed that the pre-regeneration GLC2000 land cover classification was the Cultivated and Managed land class. For regeneration areas, we used the Tropical Rainforest ecofloristic zone based on the UN FAO maps, modified with their 50% factor for disturbed vegetation categories (Ruesch and Gibbs, 2008). Areas of carbon gain were determined based on areas of gain from the regeneration map product located in the shaded cocoa areas for the 150 farms.

112.   Gibbs, H. K., and Ruesch, A. (2008). New IPCC tier-1 global biomass carbon map for the year 2000 (No. cdiac: New IPCC Tier-1 Global Biomass Carbon Map for the Year 2000). Environmental System Science Data Infrastructure for a Virtual Ecosystem.

113.   Bartholome, E., and Belward, A. S. (2005). GLC2000: a new approach to global land cover mapping from Earth observation data. International Journal of Remote Sensing, 26(9), 1959-1977.

114.   Ruesch, Aaron, and Holly K. Gibbs. 2008. New IPCC Tier-1 Global Biomass Carbon Map For the Year 2000. Available online from the Carbon Dioxide Information Analysis Center [http://cdiac.ess-dive.lbl.gov], Oak Ridge National Laboratory, Oak Ridge, Tennessee.

MORPHOLOGICAL SPATIAL PATTERN ANALYSIS (MSPA)

To calculate the number of hectares of essential habitat areas we used the Morphological Spatial Analysis (MSPA; Soille and Vogt, 2009) which takes a binary image composed of the objects of interest (foreground area) and complementary data and divides it into morphological classes, or classes that describe the spatial arrangement of habitat across the landscape. This is performed through a customized sequence of mathematical morphological operators targeted at the description of the geometry and connectivity of the image components. The MSPA segmentation results in 25 mutually exclusive feature classes which, when merged, exactly correspond to the initial foreground area. In this case, we used the binary image corresponding to loss areas from 2001 to 2019 of the Global Forest Change dataset (Hansen et al., 2013) as input.The image output had 8 classes: patch forest, outer edge, inner edge, core forest, secondary degradation, secondary deforestation, primary degradation, and primary deforestation (for definitions, see S2 File)

115.   Soille, P., and Vogt, P. (2009). Morphological segmentation of binary patterns. Pattern recognition letters, 30(4), 456-459.

116.   Hansen, M. C., Potapov, P. V., Moore, R., Hancher, M., Turubanova, S. A., Tyukavina, A., ... and Townshend, J. (2013). High-resolution global maps of 21st-century forest cover change. science, 342(6160), 850-853.

TERRABIO

To assess the shaded cocoa projects implemented by IMAFLORA, we conducted a pilot of TerraBio. TerraBio is a platform developed to provide environmental assessment and accountability to private sector firms that (1) commercialize sustainable agriculture and forest products and/or, (2) invest in sustainable business models as profitable and conservation-driven development initiatives. TerraBio directly monitors the land cover change and biodiversity measures annually to provide timely and relevant results for investment funds, businesses, and other parties invested in the success of sustainable agricultural practices (USAID, 2020). TerraBio uses a coupled approach to environmental monitoring, with the landscape component conducted with remote sensing and the biodiversity component conducted with eDNA, which work together to calculate a series of indicators. Intervention areas were shaded cocoa fields; our

counterfactual areas or 'business as usual' areas were pasture fields, and our control areas were the areas of naturally regenerated second growth forest.

117.   USAID. (2020). Private sector engagement: Mobilizing finance for Amazon biodiversity conservation. Accessed November 8th, 2022. Available online at: https://pcabhub.org/en-us/resources/fact-sheets/abf-fact-sheet.pdf

The initial set of indicators for the TerraBio pilot were derived from existing sources, including the Amazon Biodiversity Fund Brazil Key Performance Indicators (KPI; Althelia Climate Fund, 2021; USAID, 2020) and the United States Agency for International Development/Brazil's Partnership for the Conservation of Amazon Biodiversity standard indicators (USAID PCAB, 2019). These indicators were: Number of hectares directly restored; Number of hectares indirectly conserved; Carbon sequestration through revegetation; Number of keystone/priority species; Change in abundance of keystone/priority species; Change in species richness; Change in biodiversity index; Number of hectares showing improved biodiversity; and Number of hectares of essential habitat area conserved (Table 1).

118.   Althelia Climate Fund. (2021). Impact Report 2021. Available online at: https://ecosphere.plus/wp-content/uploads/2021/07/ACF-Impact-Report-2021-1.pdf. Last accessed 4-21-22.
119.   USAID Partnership for the Conservation for Amazon Biodiversity. (2019). Indicators Template. Available online at: https://pcabhub.org/en-us/monitoring-and-evaluation/2019-pcab-indicators-reporting-template-v2-english.xlsx/view. Last accessed 4-21-22.

In addition to these indicators derived from existing sources, we proposed a number of new indicators based on the literature (Cordier et al., 2021; Leese et al., 2018; Pawlowski et al., 2021). These proposed biodiversity indicators included: Alpha diversity; Beta diversity; Change in beta diversity due to the intervention; and a qualitative assessment of biodiversity change due to the intervention (Ladin et al., 2021; Drinkwater et al., 2021; Cordier et al., 2021; Jost, 2006).

120.   Pawlowski, J., Bonin, A., Boyer, F., Cordier, T., and Taberlet, P. (2021). Environmental DNA for biomonitoring. Molecular Ecology, 30(13), 2931.
121.   Drinkwater, R., Jucker, T., Potter, J. H., Swinfield, T., Coomes, D. A., Slade, E. M., ... & Rossiter, S. J. (2021). Leech blood‑meal invertebrate‑derived DNA reveals differences in Bornean mammal diversity across habitats. Molecular Ecology, 30(13), 3299-3312.
122.   Jost, L. (2006). Entropy and diversity. Oikos, 113(2), 363-375.

Table 1: Overview of all indicators calculated for the TerraBio implementation.

Indicator Type	#	Name	Calculation Method

Land Cover/ Land Use Indicator	1	Number of hectares indirectly conserved	We considered areas of stable forest within the 150 shaded cocoa farm properties provided by IMAFLORA excluding intervention sites (areas of shaded-grown cocoa) as areas that were indirectly conserved, i.e., areas that could have been deforested or degraded but were conserved due to project activities.
Land Cover / Land Use Indicator	2	Number of hectares directly restored.	We calculated the number of hectares regenerated from 2010 to 2015 within the intervention sites (areas of shaded-grown cocoa) of the 150 farms provided by IMAFLORA.
Land Cover / Land Use Indicator	3	Carbon sequestration through revegetation - net positive climate impact annually	We calculated total carbon sequestration through revegetation from 2010 through 2015 within the areas of shade-grown cocoa for the 150 farms provided by IMAFLORA.
Biodiversity Indicator (existing)	1	Number of key species due to intervention	We determined the number and identity of MOTUs associated with key species present only in shaded cocoa fields and only in pasture. We also calculated the number of species in both habitats. In addition, we used indicator species analysis to determine if there were any key species associated with either habitat.
Biodiversity Indicator (existing)	2	Change in abundance of keystone/ priority species due to interventions	We calculated key species abundance for each plot using filtered reads (Di Muri et al., 2020).
Biodiversity Indicator (existing)	3	Change in species richness due to interventions	We calculated the total species richness for each plot.
Biodiversity Indicator (existing)	4	Change in biodiversity indices due to interventions	We calculated Shannon's diversity index and Simpson's diversity index (Gloor et al., 2017).
Biodiversity Indicator (proposed)	5	Change in alpha diversity	We calculated species richness (Hill's q = 0), effective species richness (Hill's q = 1), and inverse Simpson's (Hills q = 2; Jost, 2006).
Biodiversity Indicator (proposed)	6	Beta diversity	We calculated beta diversity using Aitchison distance between sites using the Euclidean distance matrix.
Biodiversity Indicator (proposed)	7	Change in beta diversity due to interventions	We calculated Aitchison distance between treatments using the Euclidean distance matrix.
Biodiversity Indicator (proposed)	8	Qualitative assessment of change in biodiversity due to interventions	We created PCA plots to assess change in communities qualitatively.

Landscape Integrity Indicator	1	Number of hectares of essential habitat area conserved	We calculated the number of hectares of core forest, patch forest, stable inner forest edge, and stable outer forest edge (buffer zone) within the 150 shaded cocoa farm properties provided by IMAFLORA.

RESULTS

MAPPING RESULTS

According to the final disturbance mapping results, 183 hectares were degraded, and 358 hectares were deforested within the 150 IMAFLORA properties (11,546 ha) between 2010-2015. Overall, producer's and user's accuracies (± 95% confidence interval) per class from the disturbances classified map (Figure 3) are summarized in Table 2. As expected, the stable classes (stable forest and non-forest) had higher user's and producer's accuracies and lower uncertainties compared to the dynamic classes (degradation and deforestation). Nevertheless, omission and commission errors happened within and across these two groups of classes (stable and dynamic classes). For example, in the dynamic classes (degradation and deforestation): most incorrectly classified pixels in the degradation class were omitted in the deforestation class and committed to the stable forest class, and both omission and commission errors in the deforestation class happened in the degradation class. The reasons behind these errors are explained in the discussion section.

Table 2: Accuracy assessment of the disturbances map.

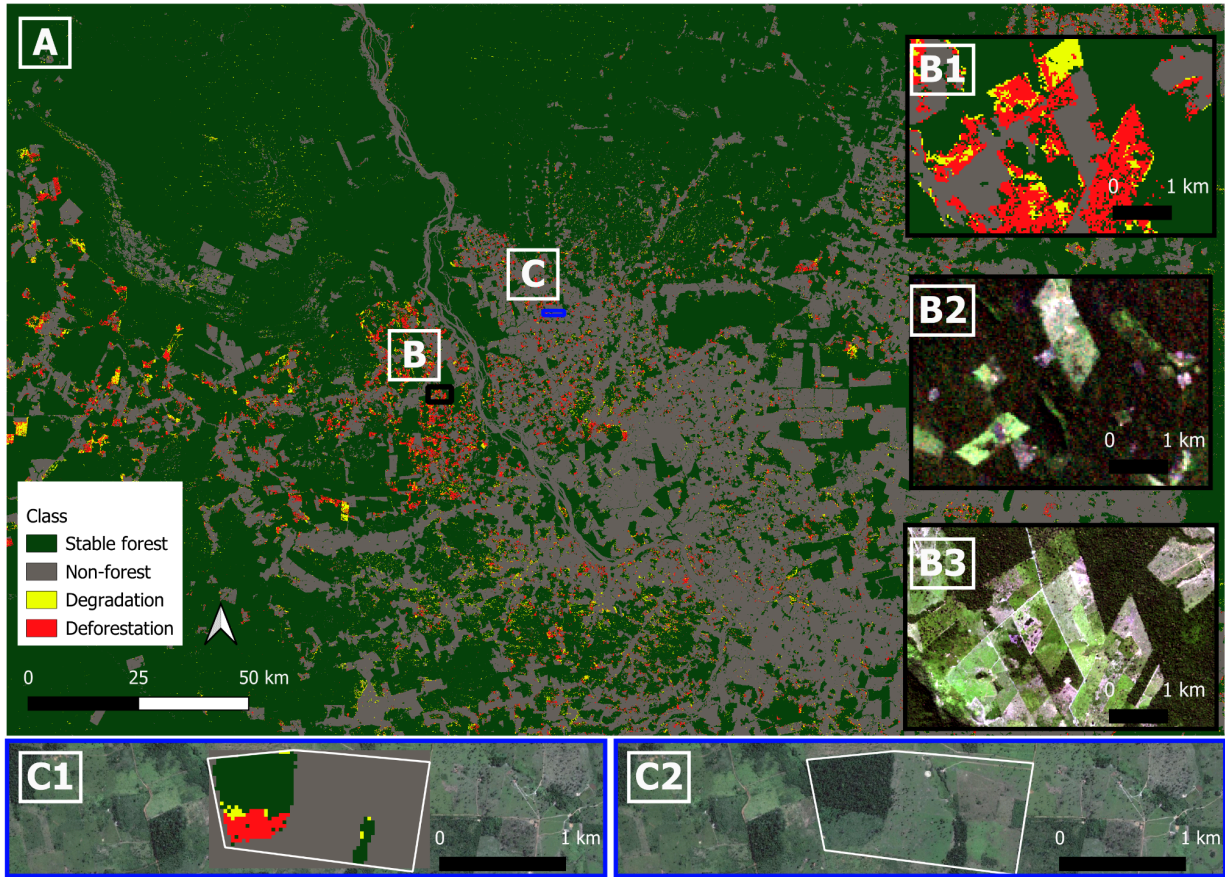| Accuracies (%)	Degradation	Deforestation	Stable forest	Non-forest
User's accuracy	40.5 ± 10.2	60.5 ± 9.1	89.0 ± 6.2	85.0 ± 5.4
Producer's accuracy	6.8 ± 3.4	30.5 ± 16.2	95.9 ± 1.8	97.0 ± 4.0
Overall accuracy	86.5 ± 4.3			

Figure 3: (A) Disturbance classification 2010-2015. Inset maps (B: B1, B2, B3) are shown with black outline and inset maps (C: C1, C2) are shown with blue outline on the main map. (B1) Inset map of disturbance classification 2010-2015 over a particular area to outline classification vs. RGB images. (B2) Inset RGB image pre-study period from Landsat 5 (July 30, 2009). (B3) Inset RGB image post-study period from Sentinel-2 (June 26, 2016). (C1, C2) Example of disturbances within one of the properties (degradation in yellow, deforestation in red, stable forest in dark green, and non-forest in gray). Note that the satellite image in (C1, C2) is from Google and does not have a timestamp associated with it.

Overall, producer's and user's accuracies (± 95% confidence interval) from the regeneration classified map (Figure 4) are summarized in Table 3. As expected, our approach performed better at identifying where regeneration did not occur compared to where regeneration did occur. The regeneration class presented low user's and producer's accuracies and higher levels of uncertainties. The biggest error source was regeneration pixels being committed to the non-regeneration class, i.e., an overestimation of areas where regeneration occurred.

Table 3: Accuracy assessment of the regeneration map.

Accuracies (%)	Non-regeneration	Regeneration

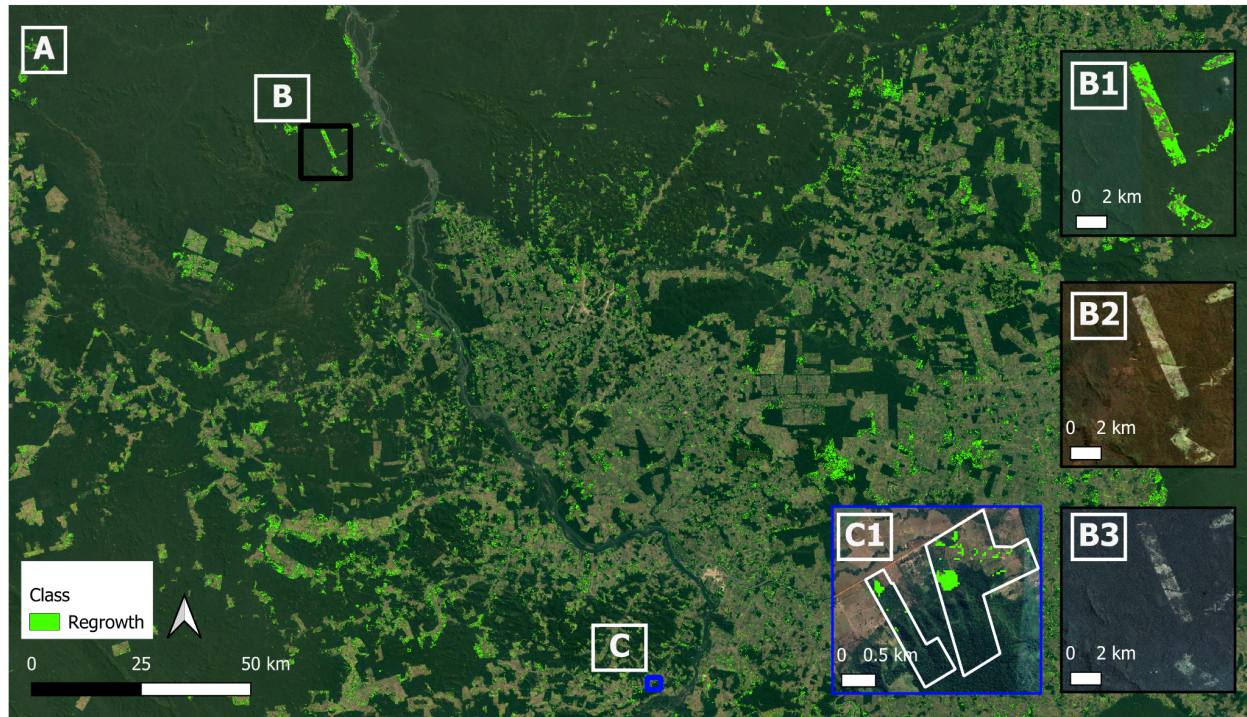|		
User's accuracy	98.3 ± 1.7	34.1 ± 9.1
Producer's accuracy	97.6 ± 0.3	43.0 ± 25.3
Overall accuracy	96.0 ± 1.6	

Figure 4: (A) regeneration map output 2010-2015. Inset maps (B: B1, B2, B3) are shown with black outline and inset maps (C: C1) are shown with blue outline on the main map. (B1) Inset map of regeneration output 2010-2015 over a particular area to outline regeneration areas vs. RGB images. (B2) Inset RGB median image pre-study period from Landsat 5 (July-August 2011). (B3) Inset RGB image post-study period from Planet NICFI mosaic (July - November 2016). (C1) Example of regeneration areas (in green) within two of the properties.

eDNA RESULTS

Overall, five cocoa sites and five pasture sites, in addition to one forest site, were surveyed by the field team. While the paired sampling design was not maintained, all but one of the fields sampled came from cluster 1 so the reduction in exogenous variation should be maintained (S3 Table).

Upon the initial bioinformatics filtering steps, a yield of 14,387,489 sequencing reads was obtained from the two sequencing runs, with 10,775,915 reads for the invertebrates dataset (5,598,018 Gillet, and 5,177,897 Zeale) and 3,611,574 for vertebrates. After applying all

taxonomic filtering steps and retrieving only target MOTUs, the number of reads retained were 2,665,095 reads for arthropods including both Gillet and Zeale primer sets, and 38,806 reads for vertebrates after the removal of human and domestic animal reads. For the vertebrates dataset, a very high proportion of human reads was detected (>75% of total reads, from 6.9 million mammal reads, 5.2 million belonged to humans), with 14 MOTUs from 10 unique families identified when considering only the target wild mammalian taxa. For arthropods, 290 MOTU from 27 unique identified families were retained after the removal of MOTUs that were not assigned any taxonomic information. Due to the low amount of reads and MOTU returned for vertebrates, further analyses focused on the arthropod dataset (S3 File).

INDICATOR RESULTS

Land Cover/ Land Use Indicator 1: Number of hectares indirectly conserved. We found 2,871 hectares within the 150 IMAFLORA properties (excluding intervention sites) were indirectly conserved between 2010-2015.

Land Cover/ Land Use Indicator 2: Number of hectares directly restored. We found 471 hectares within the intervention sites (areas of shaded-grown cocoa) of the 150 farms had regeneration between 2010-2015.

Land Cover / Land Use Indicator 3: Carbon sequestration through revegetation - net positive climate impact annually. We found carbon sequestration through revegetation in shade-grown cocoa systems in the intervention farms of 44,300 Mg C (8,860 Mg C/yr) between 2010 and 2015.

Biodiversity Indicator 1: Number of key species due to intervention. Overall, we found a total of 19 key MOTUs, including 11 in Hymenoptera and 8 in Lepidoptera. Most Hymenoptera belonged to the family Formicidae (ants), while Lepidoptera belonged to families Saturniidae and Crambidae, among others. Only one group of butterflies was detected (*Hermeuptychia hermes* [Fabricius], or the Hermes satyr).

One member of Hymenoptera was found in both cocoa fields and pasture. Five MOTU were found only in cocoa fields, including *Labidus* sp. (army ants), *Solenopsis* sp. (fire ants), *Hileithia* sp. (moth), a member of family Platygastridae and a member of Hymenoptera for which further identification was not possible. Thirteen MOTU were found only in pastures, including *Crematogaster abstinens* [Forel] and two other *Crematogaster* species, *Solenopsis geminata* [Fabricius] and another *Solenopsis* species*, Argyria* sp*., Heliura* sp., *Hermeuptychia hermes*, *Hylesia* sp. *Clepsis* sp., and a member of Hymenoptera for which further identification was not possible. No Hymenoptera or Lepidoptera were detected in forests. No species were consistently found to be indicator species.

Biodiversity Indicator 2: Change in abundance of keystone/ priority species due to interventions. As this pilot only included one time period, we compared the intervention to the

counterfactual for this one time period. We found that there was also no significant difference in key species abundance when comparing cocoa fields (intervention) and pasture (counterfactuals; Pr(>Chisq) = 0.2648).

Biodiversity Indicator 3: Change in species richness due to interventions. The mean species richness in cocoa fields was 17.9 species (SD = 11.0), while on pastures mean species richness was 18.2 (SD = 9.4). When comparing total species richness between cocoa fields and pasture, we found there was no significant difference in MOTU richness (Pr(>Chisq) = 0.978; Figure 5).
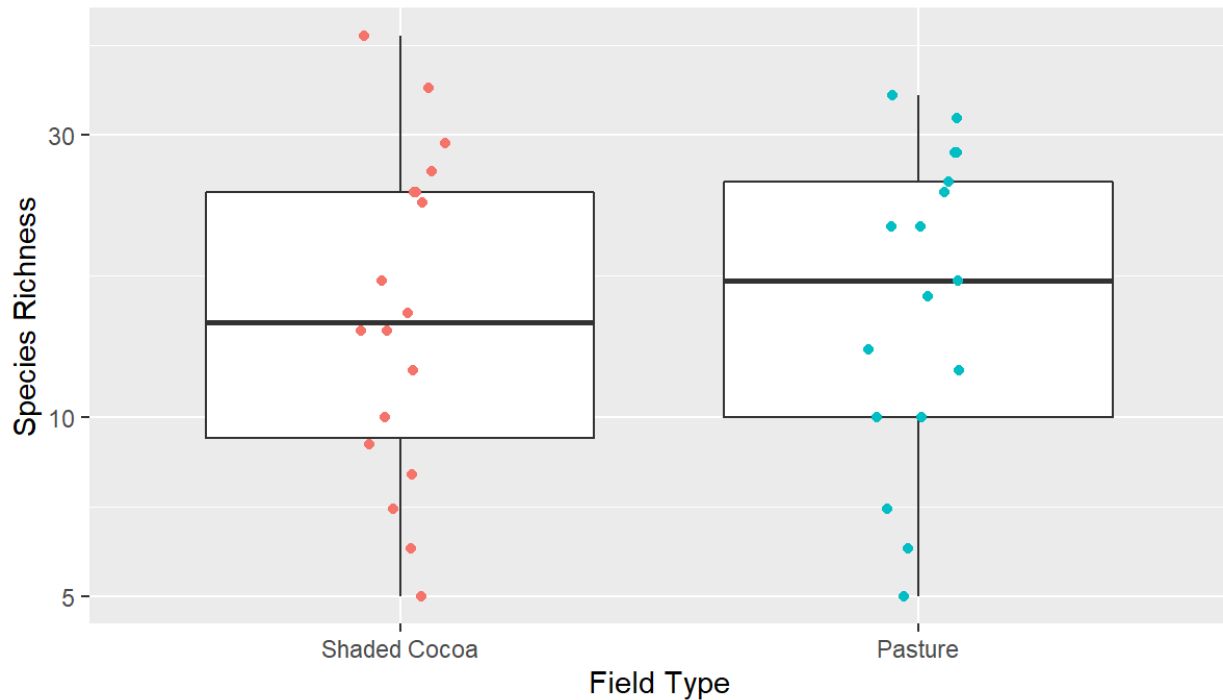
Figure 5: Species richness by field type. Each dot represents a sample point in shaded cocoa fields (red) or pasture fields (blue).

Biodiversity Indicator 4: Change in biodiversity indices due to interventions. When comparing diversity indices between shaded cocoa fields (intervention) and pasture (counterfactuals), we found that there were no significant differences for Shannon diversity (Pr(>Chisq) = 0.3639) or for Simpson's diversity (Pr(>Chisq) = 0.2964; Figure 5).
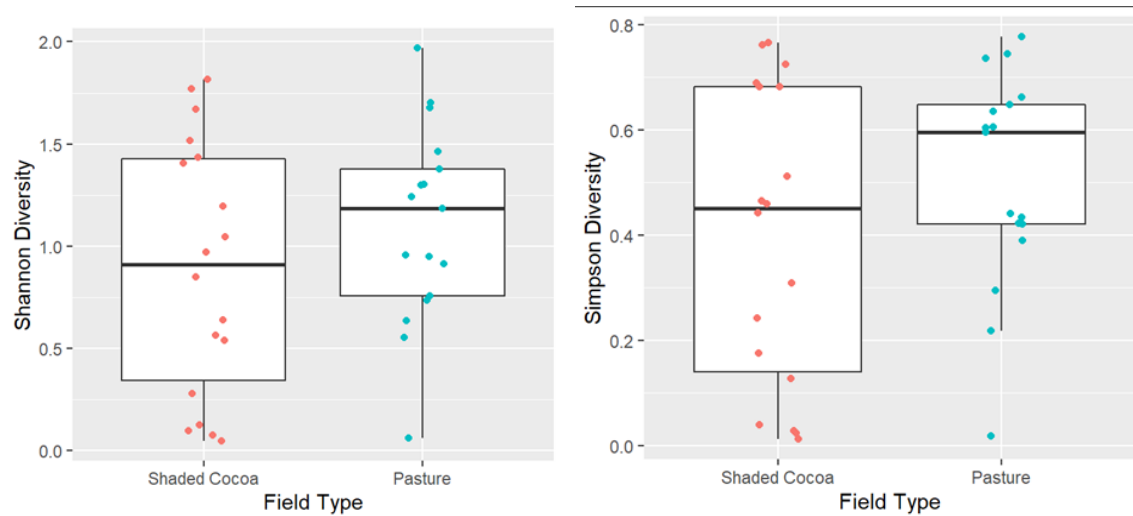
Figure 5: Shannon diversity (left) and Simpson's (right) by field type.

Biodiversity Indicator 5: Alpha diversity. When comparing diversity indices between cocoa fields (intervention) and pasture (counterfactuals), we found that there were no significant differences for species richness (Hill's q = 0; previous section), for effective species richness (Hill's q = 1; Pr(>Chisq) = 0.4824), and for inverse Simpson's (Hill's q = 2; Pr(>Chisq) = 0.5243; Figure 6).
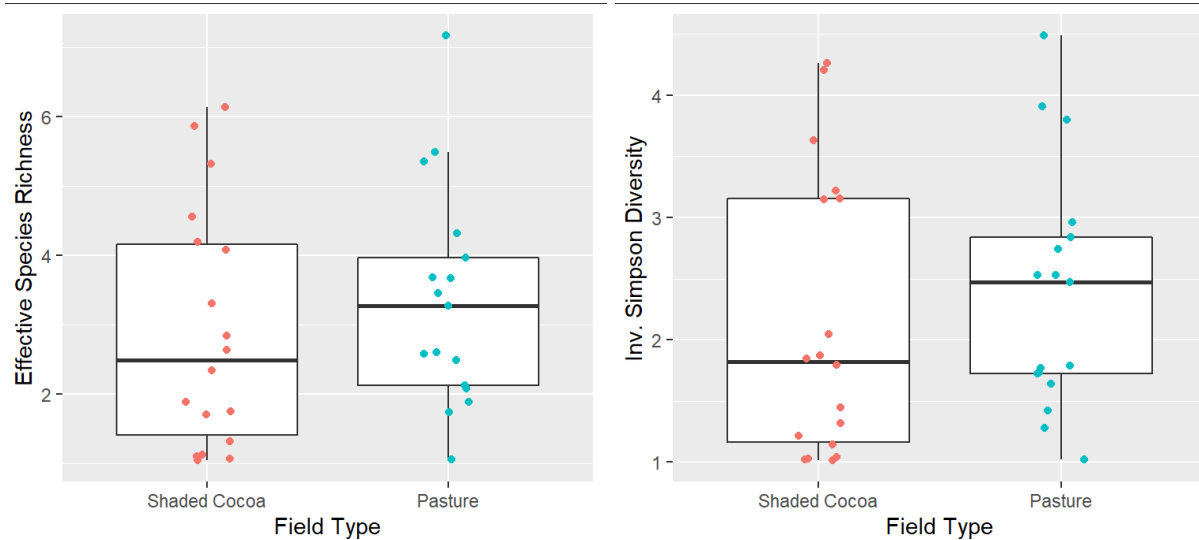

Figure 6: Effective species richness (L) and Inverse Simpson Diversity (R) by field type. Each dot represents a sample point in shaded cocoa fields (red) or pasture fields (blue).

Biodiversity Indicator 6: Beta diversity. Pairwise distances between all sampling sites ranged from 10.3 and 35.1, where 0 represents no dissimilarity, and larger distances indicate increasing dissimilarity (Figure 7). The highest dissimilarities were observed between the Cocoa 1 field and the Pasture fields.
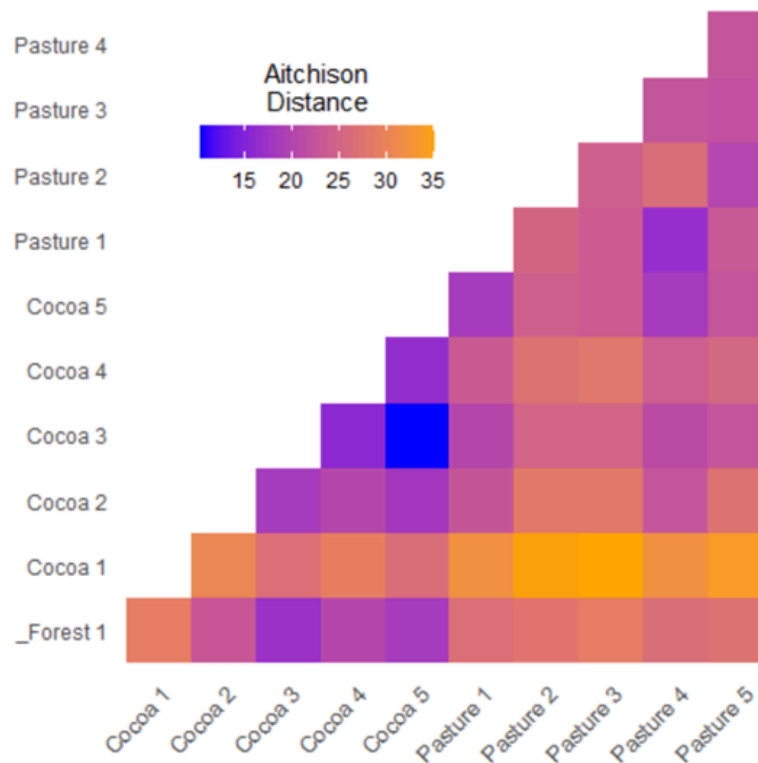
Figure 7: Beta diversity (Aitchison distance) between each site.

Biodiversity Indicator 7: Change in beta diversity due to the intervention. The Aitchison distance between shaded cocoa (intervention) and pasture (BAU) was about equal to the distance between shaded cocoa (intervention) and forest (control; 16.6 and 16.8 respectively), but much smaller than the distance between pasture (BAU) and forest (control; 23.2), though this difference was not significant (Pocock, 2006; Figure 8).

123. Pocock, S. J. (2006). The simplest statistical test: how to check for a difference between treatments. Bmj, 332(7552), 1256.
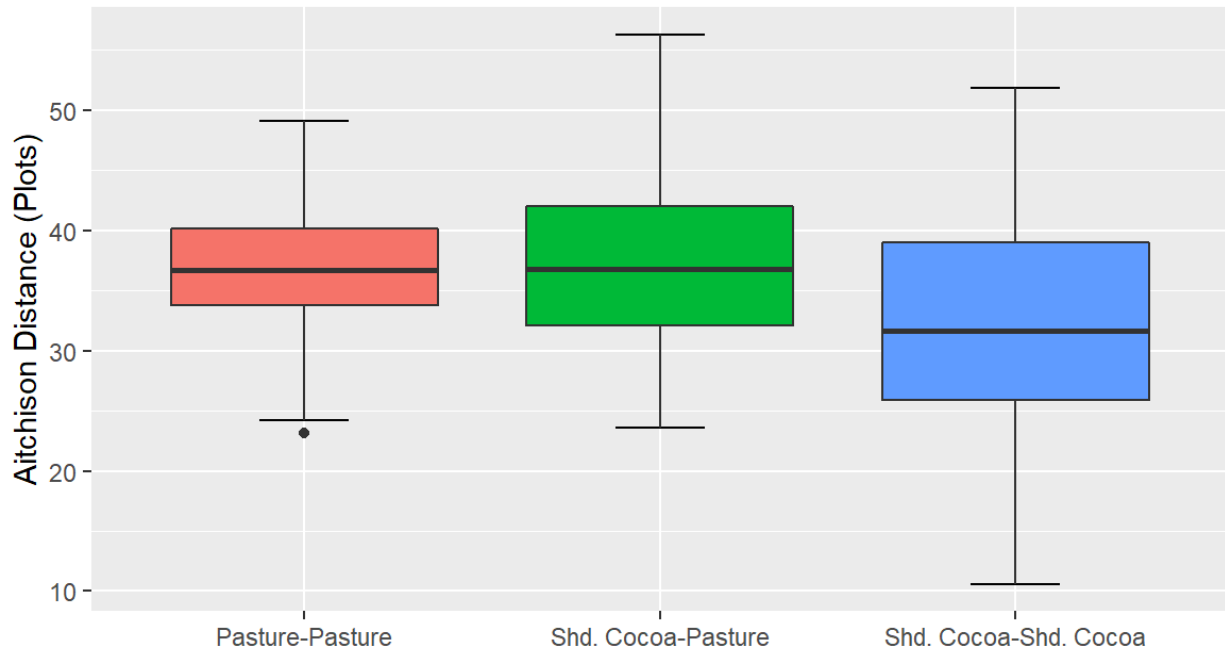
Figure 8: Aitchison distances within treatments and between treatments.

Biodiversity Indicator 8: Qualitative assessment of change in biodiversity due to the intervention. PCA graphs of plots and sites revealed that cocoa and forest sites were co-located in site-species space, while pastures were strongly separated along the first axis (Figure 9). This agrees with our findings in Biodiversity Indicator 7.
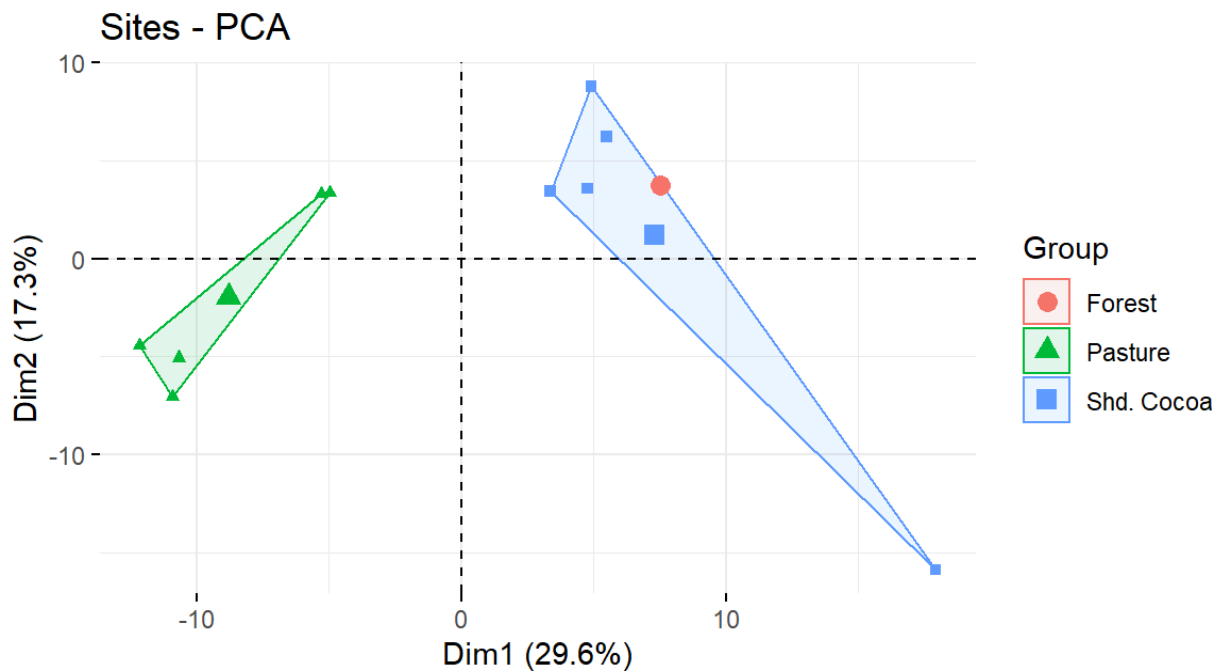

Figure 9: Arthropod PCA for arthropods found in shaded cocoa, forest, and pasture sites.

Landscape Integrity Indicator 1: No. of hectares of essential habitat area conserved.
Essential habitat areas calculated using MSPA within all farm properties with shaded cocoa included 93 ha of core forest, 114 ha of patch forest, 82 ha of stable inner forest edge, and 1,000 ha of stable outer forest edge (buffer habitat; S2 Table). Thus, a total of 289 ha can be considered critical habitat within the intervention farms, and an additional 1,000 ha is stable buffer habitat.

DISCUSSION

PILOTING THE TERRABIO PLATFORM

The pilot application of TerraBio, a coupled eDNA and remote sensing environmental monitoring platform, demonstrated the potential for such systems in monitoring sustainable agriculture such as the shaded cocoa fields implemented by IMAFLROA. Overall, our remote sensing analysis suggests that the shaded cocoa established by IMAFLORA helped revegetate over 400 hectares, and the eDNA analysis suggests that the community composition of arthropods in shaded cocoa is closer to second growth forests than that of pastures.

Based on the indicators, the broader impacts of shaded cocoa in the study area are most likely increased canopy cover, increased carbon sequestration, and more 'forest-like' habitat availability for arthropods. Land Cover/Land Use Indicators 1 and 2 both found support for the shaded cocoa indicators directly and indirectly restoring forest cover. Land Cover/Land Use Indicator 3 similarly found a net gain due to carbon sequestration by the shade canopy and cocoa in the intervention farms. While the results from the initially provided biodiversity indicators and vertebrates were inconclusive, the proposed biodiversity indicators found that arthropod communities in shaded cocoa fields were closer to forests than arthropod communities in pastures were to forests. This suggests that the habitat available to arthropods in shaded cocoa was more 'forest-like' than the pastures due to the interventions, in agreement with Landscape Integrity Indicator 1.

Importantly, our results agree with previous studies on the impacts of shaded cocoa. In Ethiopia, researchers found that shade coffee certification increased the probability of forest conservation by 19.3% (Takahashi and Todo, 2014) and that indirect forest conservation was also observed within 100m of the project areas (Takahashi and Todo, 2017). Similarly, the contribution of shaded cocoa to biodiversity conservation viewed through the lens of retaining forest-like communities echoes decades of previous research (Francesconi et al., 2013; Jha et al., 2014; Schroth and Harvey, 2007; Maney et al., 2022). Contributions of shaded cocoa systems to landscape connectivity are also well supported (Schroth and Harvey, 2007; de Jesús Cervantes-López et al., 2022), though systems emphasizing native trees are likely more successful than those using bananas and *Erythrina fusca* (Cassano et al., 2009). In Brazil's Atlantic Forest, traditional agroforests where cacao is planted under thinned native forests called *cabrucas,* have greater diversity of tree species, including forest specialist tree species, and while

they are not substitutes for undisturbed forest they do have a critical role in biodiversity conservation (Sambuichi et al., 2012).

124. Takahashi, R., and Todo, Y. (2014). The impact of a shade coffee certification program on forest conservation using remote sensing and household data. Environmental Impact Assessment Review, 44, 76-81.

125. Takahashi, R., and Todo, Y. (2017). Coffee certification and forest quality: evidence from a wild coffee forest in Ethiopia. World Development, 92, 158-166.

126. Francesconi, W., Nair, P. K. R., Levey, D. J., Daniels, J., and Cullen, L. (2013). Butterfly distribution in fragmented landscapes containing agroforestry practices in Southeastern Brazil. Agroforestry systems, 87(6), 1321-1338.

127. Jha, S., Bacon, C. M., Philpott, S. M., Ernesto Mendez, V., Läderach, P., and Rice, R. A. (2014). Shade coffee: update on a disappearing refuge for biodiversity. BioScience, 64(5), 416-428.

128. Schroth, G., and Harvey, C. A. (2007). Biodiversity conservation in cocoa production landscapes: an overview. Biodiversity and Conservation, 16(8), 2237-2244.

129. Maney, C., Sassen, M., and Hill, S. L. (2022). Modelling biodiversity responses to land use in areas of cocoa cultivation. Agriculture, Ecosystems and Environment, 324, 107712.

130. de Jesús Cervantes-López, M., Andresen, E., Hernández-Ordónez, O., Mora, F., Reynoso, V. H., and Arroyo-Rodríguez, V. (2022). Lightly-harvested rustic cocoa is a valuable land cover for amphibian and reptile conservation in human-modified rainforest landscapes. Journal of Tropical Ecology, 1-10.

131. Cassano, C. R., Schroth, G., Faria, D., Delabie, J. H., and Bede, L. (2009). Landscape and farm scale management to enhance biodiversity conservation in the cocoa producing region of southern Bahia, Brazil. Biodiversity and Conservation, 18(3), 577-603.

132. Sambuichi, R. H., Vidal, D. B., Piasentin, F. B., Jardim, J. G., Viana, T. G., Menezes, A. A., ... and Baligar, V. C. (2012). Cabruca agroforests in southern Bahia, Brazil: tree component, management practices and tree species conservation. Biodiversity and Conservation, 21(4), 1055-1077.

LEARNING FROM THE PILOT IMPLEMENTATION

Implementing the pilot allowed us to both improve TerraBio and provide guidance for other coupled monitoring platforms for the future. In general, we found multiple aspects of our approach worked well, including robust field sampling using random samples, using the management unit as the unit of analysis, and benchmarking against a control and/or business as usual land use.

LESSONS FOR REMOTE SENSING

With the remote sensing component of the coupled platform, we encountered some challenges specific to the complex agroecosystems of the Brazilian Amazon, highlighting the need for careful integration of region-specific remote sensing knowledge in developing coupled monitoring platforms. These were detected during the accuracy assessment, highlighting the importance of this step.

First, to calculate the Land Cover/Land Use and Landscape Integrity indicators, we created two map products. While the methods used to create these map products have significant support in the literature (e.g. Hunt et al., 2020; Aryal et al., 2021; Pasquarella et al., 2022), we found that applying them in this specific context had wide margins of error and some unexpected results that provided an opportunity to learn and improve upon these methods. For example, our disturbance maps, which were used for LCLU Indicator 2, showed omission errors in the stable forest class. In accordance with Chen et al. (2021), many of the omission errors associated with this class were derived from the presence of deciduous tree species ("caducifólias", in Portuguese) in this region, which show a seasonal leaf color change, leaf-off pattern (Figure 10), and changes in NDFI values. Therefore, the algorithms assume a disturbance event in forested lands, classifying most of these pixels as degradation, which explains the low accuracy obtained for these classes. Both CODED and LandTrendr should be able to capture seasonal variations of forests with varying crown covers, and parametrization for local conditions can mitigate this issue (Bullock et al., 2020a; Bullock et al., 2020b; Kennedy et al., 2018).

133. Hunt, D. A., Tabor, K., Hewson, J. H., Wood, M. A., Reymondin, L., Koenig, K., ... and Follett, F. (2020). Review of Remote Sensing Methods to Map Coffee Production Systems. Remote Sensing, 12(12), 2041.

134. Pasquarella, V. J., Arévalo, P., Bratley, K. H., Bullock, E. L., Gorelick, N., Yang, Z., and Kennedy, R. E. (2022). Demystifying LandTrendr and CCDC temporal segmentation. International Journal of Applied Earth Observation and Geoinformation, 110, 102806.

135. Chen, S., Woodcock, C. E., Bullock, E. L., Arévalo, P., Torchinava, P., Peng, S., & Olofsson, P. (2021). Monitoring temperate forest degradation on Google Earth Engine using Landsat time series analysis. Remote Sensing of Environment, 265, 112648. doi:10.1016/j.rse.2021.112648
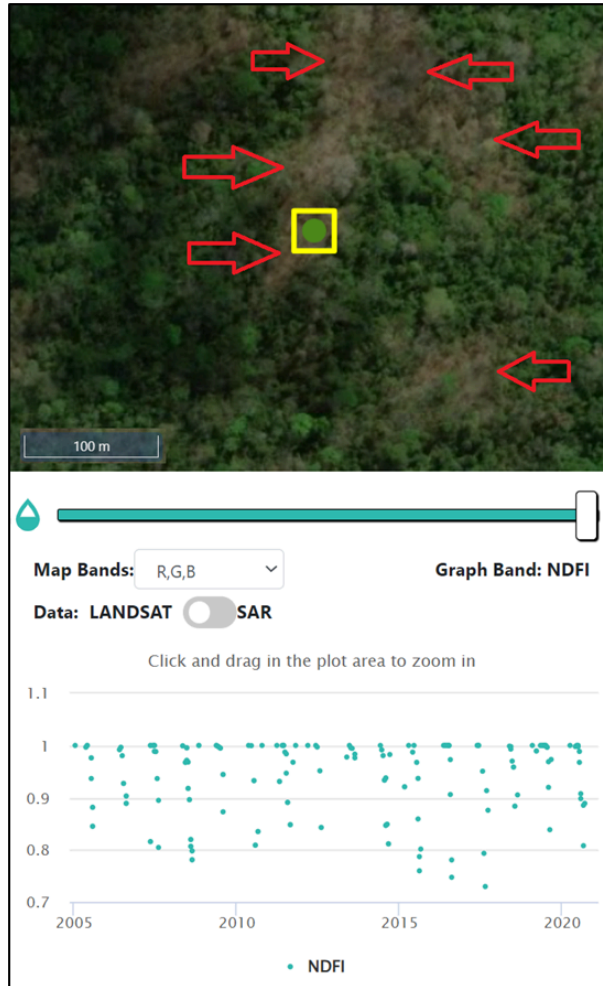
Figure 10: Plot in the CEO disturbances validation project. This pixel was misclassified as Degradation. NDFI time series show a seasonal pattern with lower NDFI values around August of each year. It is important to note that the MapBiomas classification maps these areas as "Savannic Forest Formations".

Further, it is important to fully understand the forest conversion process and pastureland management practices in the region, as this greatly influences the interpretation itself of disturbance samples for validation. Many of the samples analyzed represented patches of degraded forest cover or "dirty pastures". Some of the degraded patches presented recurrent regeneration and disturbance patterns, visible in the NDFI time series due to fragmentation or border effect (Figure 11). This is sometimes not captured in the visual interpretation analysis, where just one change event is recorded, which yields omission or commission errors for the change classes (e.g., regeneration and degradation, deforestation and degradation). For future applications, it will be important to separate the regeneration output validation from the disturbance output validation to refine confidence intervals.
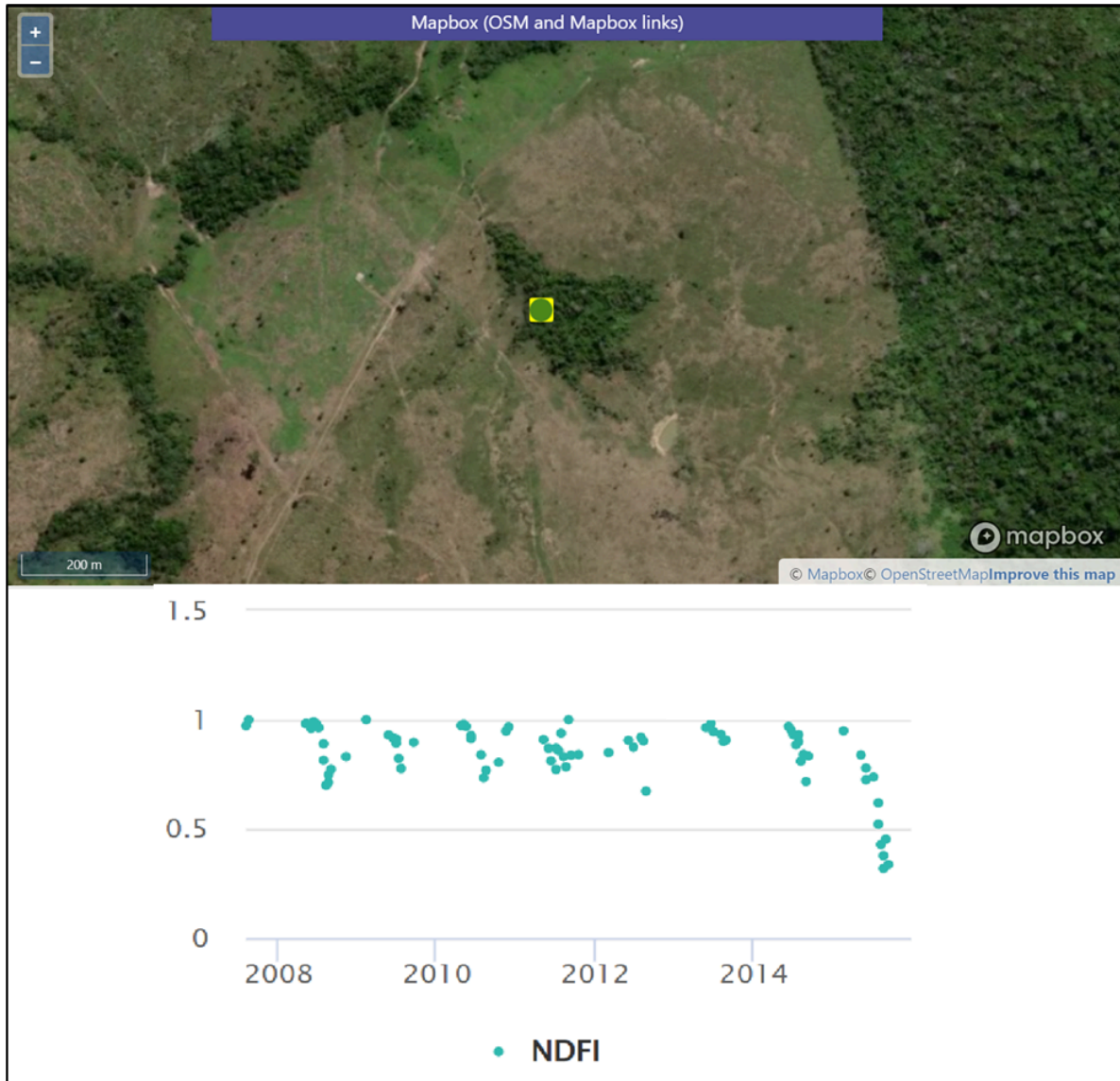
Figure 11: Example plot of the CEO change validation project showing a fragmented/degraded forested patch and its variations on the NDFI time series.

A better understanding of the degradation process and its relationship with deforestation is vital for the decision-making process of interpreting validation samples. Many times, the disturbances happen gradually, in phases, until they reach the final clear-cut stage of deforestation. The relationship between degradation and deforestation may vary significantly across the different land tenures. The same happens with the regeneration process, which will be characterized by secondary forests with different stages depending on how many years of recovery we are seeing. The interpretation of what is happening on the ground is not always clear and straightforward, especially when the interpretation is being made through satellite imagery (Figure 12). In a span of five years, we may observe two or three different events (e.g., selective logging followed by fire and then regeneration). Without the availability of Planet NICFI data prior to 2015, the

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visual inspection of these events through Landsat imagery and NDFI time series interpretation was challenging, which could have yielded validation errors.
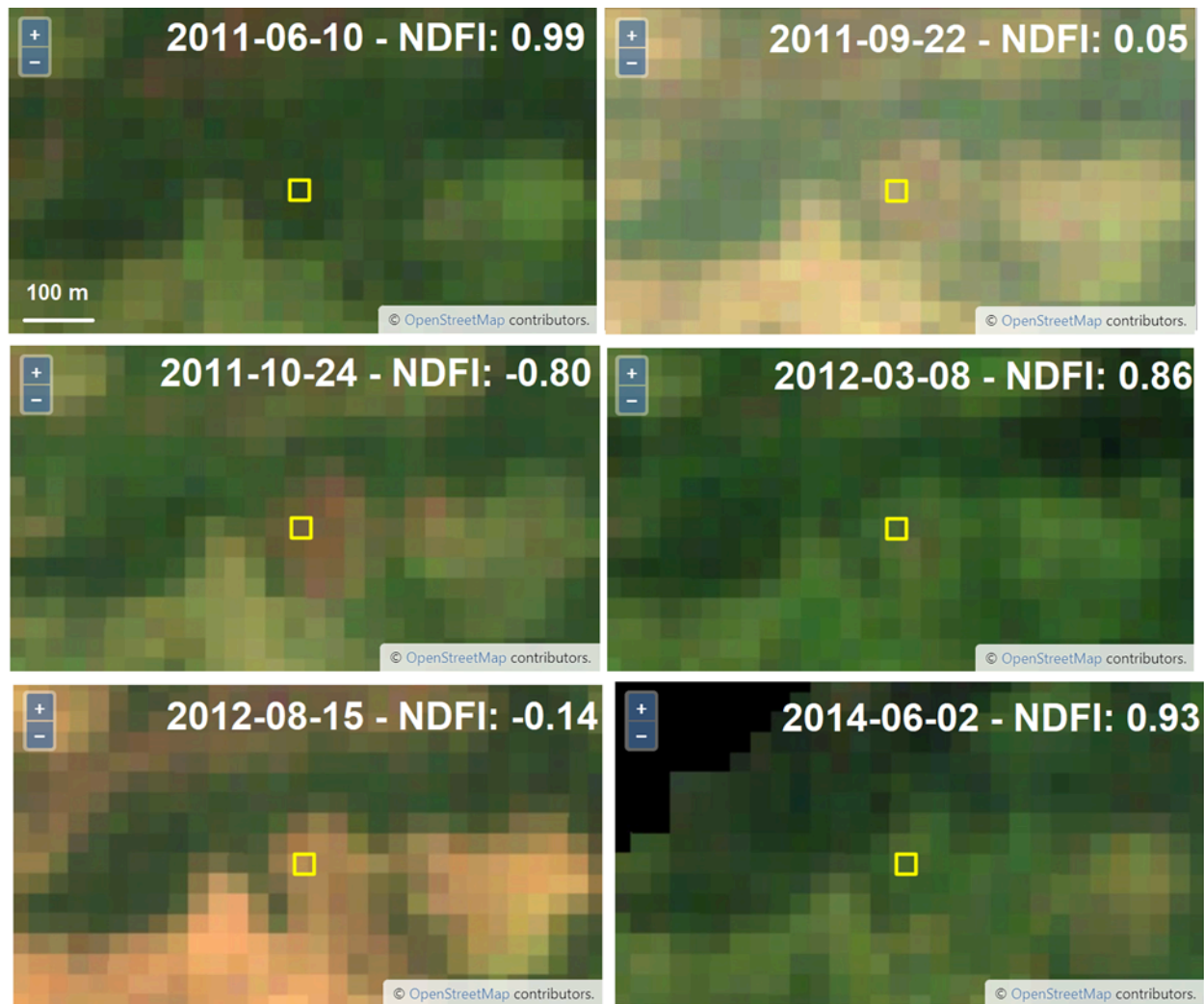

Figure 12: Example plot of the CEO change validation project showing a plot that was classified as regrowth by the algorithm, but the interpreter classified it as a single degradation event. We note some regreening between the dates and the variation in NDFI values. Although not entirely clear, the interpreter assumed selective logging followed by a fire event in 2011. Another fire event seems to have happened in 2012. It is not clear that by the end of 2014 the area was already-established pastureland.

Finally, our estimates of carbon sequestration due to revegetation do not align well with those that have been estimated previously. For example, potential sequestration for smallholder agroforestry systems like shaded cocoa has been estimated at 1.5 to 3.5 Mg per year per hectare (Montagnini and Nair, 2004). This rate suggests that we should expect approximately 6,000 Mg C sequestration due to revegetation over the study period based on our regeneration maps. Our overestimate is caused by the approach's treatment of regeneration as entirely occurring within

the study time period, which is unrealistic. For future applications of TerraBio, we plan to adapt the carbon calculation method by Bernal et al. (2018). We will use forest stand age calculated by the LandTrendr algorithm to create more accurate estimates of carbon sequestration through revegetation by more realistically accounting for the rate of growth and thus rate of sequestration.

136. Montagnini, F., & Nair, P. K. R. (2004). Carbon sequestration: an underexploited environmental benefit of agroforestry systems. In New vistas in agroforestry (pp. 281-295). Springer, Dordrecht.
137. Bernal, B., Murray, L. T., and Pearson, T. R. (2018). Global carbon dioxide removal rates from forest landscape restoration activities. Carbon balance and management, 13(1), 1-13.

We also recognize similar limitations with the MSPA calculations by using the Global Forest Change dataset. We plan to use the LandTrendr product and simplify the MSPA classes. By applying the existing LandTrendr product, and therefore, more local-based information, to both methods we expect the results to have higher accuracy, which can be supported by confidence intervals for the case of the carbon estimates.

LESSONS FOR MONITORING WITH eDNA

The success of using arthropod communities as indicators focused more on community composition points to some lessons learned for future coupled biodiversity monitoring platforms and implementations of TerraBio. The arthropod datasets did not encounter the same issues with high numbers of human reads and domesticated species, likely associated with the anthropogenic land uses (Sales et al., 2020; Strickler et al., 2015). Combined with their ecological importance, arthropods are thus a promising taxonomic group to use as a target moving forward (Latini et al., 2020; Arribas et al., 2021). Both Gillet and Zeale primers succeeded in capturing different parts of the Arthropoda phylum, however the majority of reads obtained belonged to non-target taxa, and for future studies, we recommend that both primer sets be used if both Hymenoptera and Lepidoptera groups are targeted taxa.

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biodiversity inventories: Opportunities for a site‑based genomic framework for global integration and synthesis. Molecular Ecology. 2021 Mar;30(5):1120-35.

From the reads attributed to Arthopoda, a significant fraction of detected MOTUs could not be successfully assigned at the taxonomic rank required for the downstream analyses. The lack of resolution at short fragments associated with eDNA monitoring and sparse or incomplete reference databases is a well-known issue in understudied regions such as the Neotropics (Jackman et al., 2021). For the "key species" indicators requiring identification to species, many key arthropod species were present in only one plot. Thus, when monitoring these indicators over time, as TerraBio plans to do, researchers must either use an outdated database or constantly update previous years' results in order to separate the effect of improving databases from any real biological change. Further, due to the fundamental character of eDNA data, some traditional indicators such as species abundance calculated with eDNA data can be misleading (Pawlowski et al., 2021).

141.   Jackman, J.M., Benvenuto, C., Coscia, I., Oliveira Carvalho, C., Ready, J.S., Boubli, J.P., Magnusson, W.E., McDevitt, A.D. and Guimarães Sales, N., 2021. eDNA in a bottleneck: Obstacles to fish metabarcoding studies in megadiverse freshwater systems. Environmental DNA, 3(4), pp.837-849.

This points to one key benefit of using indicators focused on overall community composition over those focused on species specific (e.g., keystone, rare, endangered, or endemic species) used elsewhere. The initial set of indicators selected for use in the pilot implementation of TerraBio were derived from existing indicators based on traditional field ecology methods where organisms are directly observed. However, eDNA represents a fundamental shift in how ecological communities are measured. Any taxonomy-based indicator requires accurate databases linking genomic sequences with their taxonomic identity (Cordier et al., 2021; Beng and Corlett, 2020). New approaches to ecological indicator selection are able to better leverage eDNA data to monitor communities using taxonomy-free approaches (Cordier et al., 2021; Leese et al., 2018; Pawlowski et al., 2021).

Our proposed indicators, chosen specifically to take advantage of eDNA data, were much more successful at identifying differences between communities found in the intervention. For example, Biodiversity Indicators 2-4 found no significant differences between the shaded cocoa and pasture sites, however, the community focused Biodiversity Indicators 6-8 all found that cocoa fields were more similar to forest plots than the pasture sites. Using these community ecology-based taxonomy-free approaches in order to fully make use of eDNA data is an important lesson learned for coupled environmental monitoring platforms, including TerraBio. However, when implementing this approach to indicators, they must be clearly communicated to stakeholders accustomed to the outputs of traditional field ecology methods. Overall, eDNA is well suited to monitoring these projects, but successful integration inmonitoring, reporting, and

verification (MRV) standards will require balancing the best available science with reporting requirements.

We also encountered some challenges with our sampling protocols when implemented in remote areas by field partners and packaging scientific best practices in a way that is easily accessible and actionable. During data collection, our field team ran into multiple practical issues while collecting soil samples using the plot sample design. Having specific areas to collect from proved very time consuming, which when added to the already long transit times between field locations resulted in reduced data collection. The size and shape of the fields also limited the field team's ability to change the location of plots if necessitated by field conditions. These challenges will become increasingly important when farm owners and community scientists are collecting data.

Thus, we suggest using a simplified larger volume sampling approach, and will be moving to this in future implementations of TerraBio. In particular, we are moving away from a plot-based approach with small amounts of soil collected to an approach collecting large volumes of soil from the entire site (1-2 L; Leempoel et al., 2020). Recent research suggests that large soil volumes are likely needed to accurately capture community representation (e.g., Cantera et al., 2019; Holmes, 2021; Sakata et al., 2021; Leempoel et al., 2020). This sampling approach will also be significantly easier for farmers and community scientists to implement, and early testing is promising (unpublished data).

142.   Leempoel, K., Hebert, T., and Hadly, E. A. (2020). A comparison of eDNA to camera trapping for assessment of terrestrial mammal diversity. Proceedings of the Royal Society B, 287(1918), 20192353.
143.   Cantera, I., Cilleros, K., Valentini, A., Cerdan, A., Dejean, T., Iribar, A., ... and Brosse, S. (2019). Optimizing environmental DNA sampling effort for fish inventories in tropical streams and rivers. Scientific Reports, 9(1), 1-11.
144.   Holmes, V. (2021). Environmental DNA Monitoring of Non-Native Mudpuppy (Necturus Maculosus) and Transient Rainbow Smelt (Osmerus Mordax). The University of Maine.
145.   Sakata, M. K., Watanabe, T., Maki, N., Ikeda, K., Kosuge, T., Okada, H., ... and Minamoto, T. (2021). Determining an effective sampling method for eDNA metabarcoding: a case study for fish biodiversity monitoring in a small, natural river. Limnology, 22(2), 221-235.

Further, during our data analysis, we noticed that fewer reads were returned for cocoa fields than for pasture fields, with multiple plots missing data entirely or exhibiting very low species richness (i.e., number of detected species, S3 Table). Differences in soil moisture may have accounted for this result, as pastures were generally drier than shaded cocoa fields and forests. If the desiccant volume was not sufficient to handle higher soil moistures, then sample degradation may have occurred unevenly between the more shaded and thus wetter cocoa and forest samples and the drier pasture samples (Strickler et al., 2015). We will be testing higher volumes of

desiccant and Longmire's solution, as many of the sustainable agricultural projects occur in remote areas and tropical or sub-tropical climates (Guerrieri et al., 2020; Williams et al., 2016). Early results suggest this approach may help with high moisture soils, allowing for more accurate comparisons between land uses (unpublished data).

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CONCLUSION

Platforms that directly monitor land cover change and biodiversity on an annual basis by coupling remote sensing and environmental DNA (eDNA) can provide timely and relevant results for parties interested in the success of sustainable agricultural practices. Monitoring information collected from these platforms serves two essential functions: assessing the effectiveness of project-level management actions and approaches and facilitating ongoing learning about the circumstances in which different approaches outperform others.

In this pilot, we found that shaded cocoa projects implemented by IMAFLORA contributed to forest and biodiversity conservation in the Brazilian Amazon. The broader impacts of shaded cocoa projects in the study area, as revealed through the indicators, are most likely increased canopy cover, increased carbon sequestration, and more 'forest-like' habitat availability for arthropods. Following the successful pilot application of TerraBio to assess the effect of shaded cocoa interventions near São Félix do Xingu, in Pará, Brazil on biodiversity conservation, the platform will be expanded to other projects created through innovative funding mechanisms.

Implementing this pilot allowed us to provide guidance for coupled monitoring platforms for the future, including TerraBio. In general, we found multiple aspects of our coupled approach worked well. These included our sampling design using randomly selected farms and using the management unit as the unit of analysis. Benchmarking the intervention against a control and business as usual land use was a unique and cost-effective way to validate results on the project level while controlling for exogenous variables. Using remote sensing and eDNA data collection in tandem to calculate indicators also provided a more holistic view than either alone.

For our remote sensing analysis, we found that detecting forest disturbances and regeneration was challenging due to regional land management practices and vegetation characteristics, suggesting better algorithm parametrization to local conditions is needed to improve future accuracy. Understanding the degradation process and its relationship with deforestation is also vital for interpreting validation samples. Additionally, more realistic approaches to forest growth

for carbon calculation analysis are needed. For our eDNA analysis, we suggest moving to straightforward sampling designs using high volume sampling and replication. We also found that taxonomy-free arthropod community focused indicators were more successful at illuminating ecologically holistic differences between intervention (shaded cocoa) and business as usual (pasture) scenarios.

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