Evaluating Soil Health Indicators across a Restoration Gradient in Montane Cloud Forests in the Cordillera de Talamanca, Costa Rica

BSc thesis by Sebastiaan de Kraker

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Abstract

Tropical montane cloud forests (TMCF) are ecologically and hydrologically significant ecosystems that have suffered widespread conversion to agricultural land, particularly pasture. Ecosystem restoration of degraded TMCF has become a priority, because of the ecosystem services it provides, but assessing whether restoration is progressing requires reliable indicators of soil health and ecosystem functioning. This study evaluated earthworm abundance and biomass, other macrofaunal biomass, and selected soil properties (bulk density, volumetric soil moisture content and soil pH) as indicators of restoration progress across a land-use gradient at Cloudbridge Nature Reserve in the Cordillera de Talamanca, Costa Rica. Four land-use types were compared: pasture, reforested forest, natural regrowth and old-growth forest, the latter serving as the ecological reference condition.

Earthworm abundance and biomass differed significantly among land-use types but showed a counterintuitive pattern along the restoration gradient, with the highest values in pasture and reforested plots and near zero counts in natural regrowth and old growth forest. This non-linear response is attributed to differences in organic matter quality, litter chemistry and land-use history rather than restoration status, consistent with findings from other tropical restoration studies. Other macrofaunal biomass did not differ significantly across land-use types. Visual classification indicated that the large majority of earthworms were endogeic, consistent with the dominance of this functional group in humid tropical soils. Bulk density showed the strongest and most consistent pattern, decreasing significantly from pasture through reforested forest to natural regrowth and old growth, with significant pairwise differences between all land-use pairs except natural regrowth and old growth. Volumetric soil moisture content differed significantly overall but no specific pairwise differences survived Holm correction, and soil pH showed no significant differences, partly attributable to the limited resolution of the colorimetric measurement method used. Significant positive correlations were found between earthworm abundance and biomass and bulk density, but scatter plot inspection revealed these to be driven by land-use type as a confounding variable rather than a direct causal relationship.

Bulk density emerged as the most reliable soil health indicator across the restoration gradient, offering a sensitive and practically measurable reflection of soil physical recovery with direct implications for the recovery of water infiltration capacity and the water regulating ecosystem service of montane cloud forests. Earthworms showed value as indicators of land-use transition but not as standalone indicators of restoration progress given their non-linear successional response.

Contents

Introduction

Costa Rica's conservation efforts and pressures

Costa Rica is globally recognised for its tropical forests, biodiversity and successful conservation policies. However, the country suffered one of the world's highest deforestation rates during the previous century, with its climax around 40 years ago in the 1980s when the forest cover reached its minimum of 21% (de Camino Velozo et al., 2016). Key environmental policy reforms were introduced since, with the most crucial one being the Forestry Law 7575 in 1996 (de Camino Velozo et al., 2016; Ibarra Gené, 2007). This law included the prohibition of Land-Use Change (LUC) of forested areas into agriculture or cattle farming as a way to claim property and the pioneering institutionalizing of Payment for Environmental (ecosystem) Services (PES). Since, successes were made and forest cover is estimated at 52% of terrestrial Costa Rica, half of this is within the national protected areas system and the other half is privately owned (de Camino Velozo et al., 2016). The PES also has its limitations, the rates are hard to receive and often too low, therefore other land-uses are often more economically lucrative for landowners (de Camino Velozo et al., 2016; Ibarra Gené, 2007). So despite the gains, landowners often rather engage in other land-uses and as the population and urban areas continue to grow and expand, the landscape is disrupted and pressure is put on the environment and related ecosystem services(Ibarra Gené, 2007; Rodríguez-Bonilla & Quesada-Román, 2026). The change and type of land-use have effect on the ecosystem and its services and different land-uses have different effects. LUC often changes the physical and chemical characteristics of the land and consequently affects the hydrological properties of the ecosystem and hydrological cycle (Hasan et al., 2020; Jin et al., 2015).

TMCF importance and hydrological cycle

Within Costa Rica's recovering but pressured forest landscape, not all forest types carry equal ecological significance and value of ecosystem services. Tropical montane cloud forests (TMCF) are considered among the most biologically diverse and richest ecosystems on earth, recognised as hotspots of species endemism, and play a critical role in the regulation and provisioning of water, regional climate and carbon storage (Muñoz-Villers et al., 2015; Salinas et al., 2021). According to Bruijnzeel et al. (2011) the fee paid by downstream users under PES schemes is mostly for the hydrological services provided by the montane cloud forest (MCF). Cloud forests help prevent landslides and surface erosion while simultaneously reducing flood risk during heavy rainfall events in the wet season and sustaining high base flow during the dry season in downstream areas (Bruijnzeel, 2001; Foster, 2001; Muñoz-Villers et al., 2015). These hydrological services, water availability, flood control and water conservation, are categorised within the water regulating ecosystem service (Hasan et al., 2020). What makes cloud forest hydrology distinct from other montane forests is the input of fog, or horizontal precipitation, to the water balance from clouds moving through the forest canopy (Bruijnzeel, 2001; Holder, 2005). Fog precipitation occurs when cloud droplets are intercepted on surfaces such as foliage and wood, which act as fog filters (Holder, 2005). This interception is further enhanced by epiphytes, which may represent up to a quarter of all plant species in cloud forests and are often highly endemic (Foster, 2001). Through the capture and slow release of atmospheric moisture, epiphytes contribute to the water regulation function of the forest, with storage capacities reported between 3,000 and 50,000 litres per hectare (Richardson et al., 2000; Sugden, 1981), while also storing a significant proportion of the canopy nutrient pool (Foster, 2001; Richardson et al., 2000).

Threats of LUC and climate change

These same characteristics that make cloud forests hydrologically valuable also make them exceptionally vulnerable. Between 1981 and 1990, tropical montane forests were lost at faster rates than lowland tropical forests such as the Amazon, and by around 2000 roughly 45–55% of cloud-affected forests had been converted to other land uses (Bruijnzeel et al., 2011). Historically, MCF have experienced deforestation on a large scale, and it is estimated that around half of the tropical biome is currently in some stage of recovery from anthropogenic activities (Silver et al., 2000). In recent years, climate change has become an increasing threat to the hydrological cycle and functioning of MCF, in particular through changes in cloud formation (Bruijnzeel et al., 2011; Silver et al., 2000). As the climate warms, the atmosphere becomes more humid because rising sea surface temperatures increase evaporation, which reduces how quickly air cools with height (Foster, 2001). This shifts the cloud belt upward, shrinking the zone of cloud immersion that MCF depend on, This process is already being observed in the Monteverde region of Costa Rica (Foster, 2001). Because the altitude band of cloud formation is narrow, MCF occur in fragmented strips and are therefore particularly sensitive to such shifts. Climate change is expected to upset the dynamics of cloud forests, causing biodiversity loss in both flora and fauna, altered community structure and possibly forest death, which in turn will impact the hydrological cycle and degrade the ecosystem and its services (Foster, 2001). Epiphytes, which rely entirely on atmospheric moisture and occupy narrow microhabitats, are highly sensitive to changes in humidity and cloud immersion, making them vulnerable early indicators of these impacts (Foster, 2001).

Effects of LUC on hydrological cycle and soil

Alongside climate change, direct conversion of cloud forest to agricultural land remains the primary driver of ecosystem degradation in MCF, fundamentally altering the hydrological cycle (Bruijnzeel, 2001; Bruijnzeel & Hamilton, 2000; Muñoz-Villers et al., 2015). Without the forest canopy, fog precipitation is no longer intercepted by vegetation. As previously discussed, this input can contribute significantly to the water balance of the cloud forest. The removal of this mechanism does not necessarily mean that the water bypasses the system entirely, but it does mean that the input unique to cloud forests, also supporting the cloud forest endemism, and the buffering effect it provides on streamflow and dry season water availability, is lost (Muñoz-Villers et al., 2015). Besides vegetation, LUC also alters the physical and chemical characteristics of the soil and consequently its hydrological properties, affecting erosion resistance, water storage, drainage and filtration, together they influence the water holding capacity of the system and water is mainly held in the soil (Foster, 2001; Hasan et al., 2020; Jin et al., 2015; Veldkamp et al., 2020). (Foster, 2001) uses the simplified 'Bucket model' to illustrate how different land-uses affect the water balance as shown in Figure 1. In this model, the soil functions as an open bucket that absorbs rainfall, prevents flooding and slowly releases water as dry weather flow to downstream areas. The amount of water entering the bucket differs between land-use types, Figure 1 illustrates these relationships. The cloud forest bucket is the fullest due to the combined effect of additional fog precipitation input and reduced evapotranspiration. The pasture bucket, while lacking fog input, retains more water than standard montane forest due to lower transpiration by grass cover. The montane forest bucket is the emptiest, it lacks fog precipitation while having the highest evapotranspiration of the three systems.

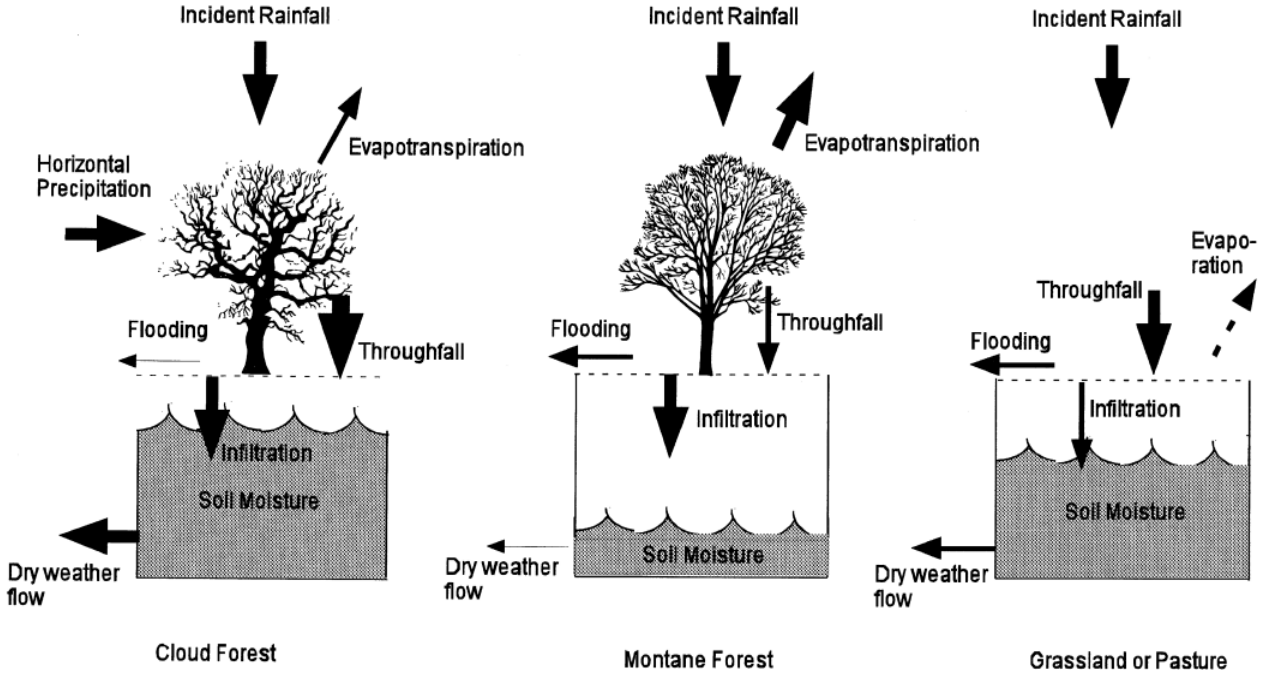

Figure 1: Water bucket model by Foster (2001), representing three ecosystems and their simplified water balance

This model illustrates how cloud forest conversion to pasture fundamentally alters the water balance, reducing the buffering capacity that sustains dry season baseflow and flood prevention downstream (Foster, 2001). The study of (Muñoz-Villers et al., 2015) on MCF conversion in Mexico to pasture or crops found an increase of 10% to 15% in annual streamflow after conversion, however a 50% lower baseflow was observed at the end of the dry season, mainly due to lower evapotranspiration rates. This was also associated with increases in overland flow in combination with lower soil infiltration (due to soil compaction caused by grazing), flooding may become more common and more intense and will lead to soil erosion, these finding support the water bucket model presented by Foster, (2001). Beyond hydrology, deforestation changes most of the soil properties and the soil functions are therefore degraded, it leads to lower soil organic carbon stock, higher soil bulk density and changes in soil pH (Veldkamp, 1994). These changes are experienced most rapidly in the organic-matter rich top soil where the biological activity is highest, in deeper layers of the soil these changes occur slowly and can take several decades (Veldkamp et al., 2020).

Soil degradation and restoration

Because water is mainly held in the soil, the degradation of soil functioning is central to the loss of water regulation capacity following land-use change. The beneficial effects of soil on water intake capacity, provided by high organic matter content and abundant faunal activity, may persist for around two years after clearing, but exposure of the soil surface to the elements leads to rapid decline thereafter (Bruijnzeel, 2001). Reversing this degradation is the aim of

ecosystem restoration, defined as the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed, towards an appropriate reference model (Gann et al., 2019). Recovery itself refers to the trajectory along which an ecosystem regains its structure, composition and functioning, and may occur passively through natural regeneration or be actively assisted through intervention (Gann et al., 2019). Determining whether such recovery is actually taking place, requires measurable indicators that reflect changes in ecosystem functioning over time rather than vegetation structure alone.

Macrofauna and earthworms as indicators

Soil macrofauna are well suited to this purpose. They play a critical role in determining soil bulk density and porosity and are consequently considered essential components of soil quality (Ruiz et al., 2011), and their abundance and diversity have been proposed both as bioindicators of ecosystem health (Muys & Granval, 1997; Paoletti, 1999) and as indicators of the degree of ecosystem restoration (Dunger & Voigtländer, 2005; Majer et al., 2007; Snyder & Hendrix, 2008). More broadly, the burrowing activity of soil macrofauna has a positive effect on soil macroporosity, air permeability, water infiltration capacity and water holding capacity (Schon et al., 2021), directly linking macrofaunal activity to the water regulating functions described above. Among the macrofauna, earthworms are of particular importance, as they significantly influence soil erosion and nutrient transfer throughout ecosystems (Jouquet et al., 2014), and because of the services they provide and their ability to alter their physical environment they are considered ecosystem engineers (Jouquet et al., 2006; Keith & Robinson, 2012). Earthworms are commonly divided into three broad functional groups, each with a different influence on key soil processes (Keith & Robinson, 2012). Epigeic species dwell in the leaf litter and topsoil and mainly facilitate the breakdown of litter and organic material. Anecic species also contribute to decomposition, but their greatest influence is on soil porosity, as they burrow deep vertical tunnels that enhance water infiltration. Endogeic species play a key role in the formation of soil aggregates, their burrowing also increases porosity, though they tunnel more horizontally, and nutrients are released through their casts (Keith & Robinson, 2012). The disruption of the soil macrofaunal community through deforestation and pasture establishment therefore has direct consequences for soil functioning. Importantly, the response of macrofauna to restoration is not always straightforward: while the abundance of many macroinvertebrate groups increases with forest regeneration, this pattern is not universal (Veldkamp et al., 2020), and earthworms have been observed to decrease in abundance in successional forests relative to pastures (Zou & Gonzalez, 1997). Factors contributing to this decline may include alterations in soil moisture content, soil pH, and the quantity and chemistry of litter inputs (Zou & Gonzalez, 1997). Although reforestation reverses many of the effects of deforestation, recovery can take decades and soil properties often continue to differ from those of natural forests (Veldkamp et al., 2020).

Soil health and indicators

Given that both soil properties and soil macrofauna respond measurably to land-use change, they offer practical tools for tracking whether degraded land is recovering. Assessing whether restoration is progressing requires measurable indicators that reflect changes in ecosystem functioning over time (Gann et al., 2019). One way to assess restoration progress is through the evaluation of soil health, defined as the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals and humans (Lehmann et al., 2020). Most soil ecosystem functions are difficult to assess directly and are therefore measured from soil properties, covering physical, chemical and biological characteristics (Muñoz-Rojas, 2018; Zornoza et al., 2015). Physical indicators such as bulk density and soil moisture content reflect the capacity of the soil to store and discharge water, while chemical indicators such as soil pH influence nutrient availability and biological activity, both among the most widely used indicator categories in restoration studies (Gatica-Saavedra et al., 2023). Biological indicators, particularly soil macrofauna such as earthworms, play a critical role in determining soil bulk density and porosity and are considered essential components of soil quality (Ruiz et al., 2011). Their abundance and diversity have been proposed as bioindicators of ecosystem health (Muys & Granval, 1997; Paoletti, 1999). Therefore, this study evaluates the extent to which soil macrofauna

and soil properties can serve as soil health indicators of restoration progress and the recovery of ecosystem functioning, particularly water regulation, across a restoration gradient in a tropical montane cloud forest.

General Research Question (GRQ):

How do macrofauna, with a focus on earthworms, and soil properties vary across a restoration gradient in a tropical montane cloud forest, and to what extent can they serve as soil health indicators of ecosystem restoration progress?

Specific Research Questions (SRQ):

1. How do earthworm abundance, biomass and other macrofaunal biomass differ across land-use types?
2. How do soil properties (bulk density, soil moisture content and soil pH) differ across the land-use types?
3. What relationships exist between earthworm abundance and biomass and the measured soil properties?
4. To what extent do soil macrofauna and soil properties serve as indicators of restoration progress across the restoration gradient?

Background chapter

Cloudbridge Nature Reserve

Cloudbridge Nature Reserve is a private nature reserve established in 2002 when Ian and Genevieve Giddy purchased a 60-hectare cattle farm bordering Chirripó National Park in southern Costa Rica. The reserve was subsequently extended through the purchase of six additional small cattle farms and now covers approximately 220 hectares (Cloudbridge Nature Reserve, 2024a, 2024d). The reserve functions as a biological corridor, connecting Chirripó National Park to the east with other protected areas and forested land to the west, enabling the movement of wildlife such as jaguars, tapirs, pumas and the resplendent quetzal between otherwise fragmented forests (Cloudbridge Nature Reserve, 2024b). Its name reflects both the ecological and meteorological character of the site with warm moist air masses moving inland from the Pacific Ocean rise along the Talamanca mountains each afternoon and condense into cloud cover, while the reserve's reforestation work has restored the forested 'bridge' that allows fauna to migrate between north and south (Cloudbridge Nature Reserve, 2024d).

Reforestation

Reforestation at Cloudbridge began in 2002 in collaboration with local and international experts, with the goal of returning degraded agricultural land to restored montane cloud forest (Cloudbridge Nature Reserve, 2024d). With one exception, all trees planted are native to the surrounding high-altitude forest of the Talamanca mountain range, most bearing fruit and seeds to encourage the return of birds and animals (Cloudbridge Nature Reserve, 2024a). Initial efforts involved planting large hillsides with native pioneer species such as *Cecropia spp.* and fast-growing alder (*Alnus acuminata*), alongside a small number of climax species. Early planting was challenged by invasive grasses, and survival rates improved significantly once techniques shifted toward planting older, more established seedlings with targeted maintenance and mulching (Cloudbridge Nature Reserve, 2024c). Seeds of 12 to 16 pioneer and climax species were collected from old-growth forest and propagated in the reserve's nursery for six months to two years before planting. In areas where conditions allowed, natural regeneration was permitted to proceed without active planting

(Cloudbridge Nature Reserve, 2024c). The progress of reforestation at Cloudbridge can be seen in Figure 2, showing the same area as pasture land in 2002 and as recovering secondary forest 22 years later in 2024.

Figure 2: Left: pasture land as seen from Cloudbridge's main trail in 2002. Right: the same area in 2024 after 22 years of reforestation. Source: Cloudbridge Nature Reserve (2024c); Sluiter (2025)

The reserve therefore contains a mosaic of land-use types at different stages of recovery, four of which were selected for this study, these are described in detail in the methodology.

Methodology

Study area

The study was conducted at Cloudbridge Nature Reserve in San Gerardo de Rivas, Costa Rica, within tropical montane cloud forest. The reserve is situated in the Cordillera de Talamanca, which runs from central Costa Rica into western Panama, separating the Atlantic and Pacific watersheds. Cloudbridge lies within the Río Chirripó Pacífico catchment and spans an elevation range from approximately 1,550 m at the entrance to 2,600 m at its highest point (Cloudbridge Nature Reserve, 2024a). Based on elevation, the study area falls within the lower montane cloud forest zonation of the Cordillera de Talamanca, at the lower boundary of the montane forest belt described by (Kappelle et al., 1995).

Weather data from the San Gerardo de Rivas meteorological station (IMN, 2026), located within the pasture plot of the study area, indicate a mean daily temperature of 17.4°C and mean relative humidity of 92.7% over the period January 2025 to June 2026, with total recorded rainfall of 4,747 mm across 533 days. Monthly rainfall totals show a clear seasonal pattern, with the driest months from January to March and peak rainfall in May–June and October (Figure 3). This is consistent with the climate of the Pacific slope of the Cordillera de Talamanca, characterised by a dry period from December to April and the most intense rainfall between May and November (Cloudbridge Nature Reserve, 2024a). The location of the study area and the four land-use types along the El Jilguero trail are shown in Figure 4.

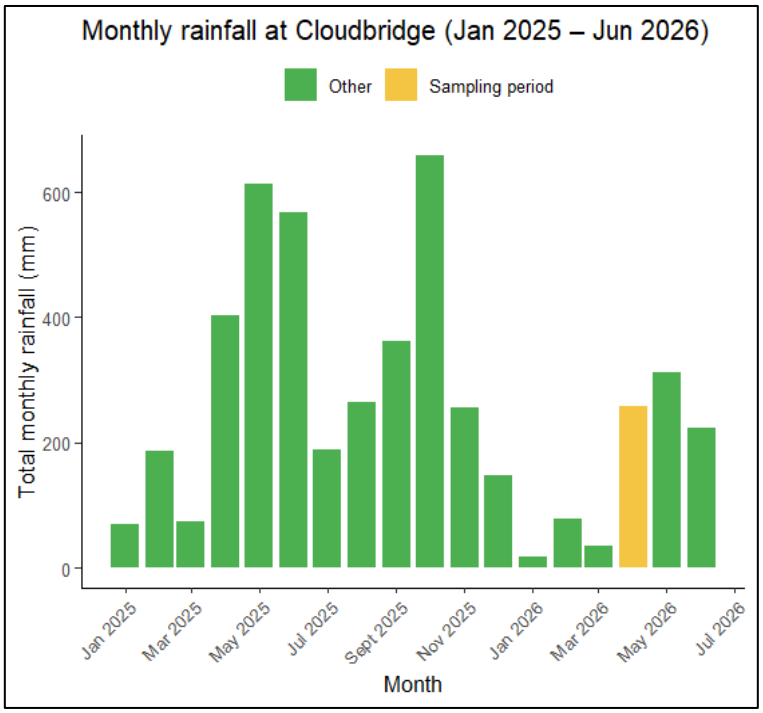

Figure 3: Monthly rainfall from (Jan 2025-June 2026), with in yellow rainfall during the sampling month (April 2026). Source: IMN (2026)

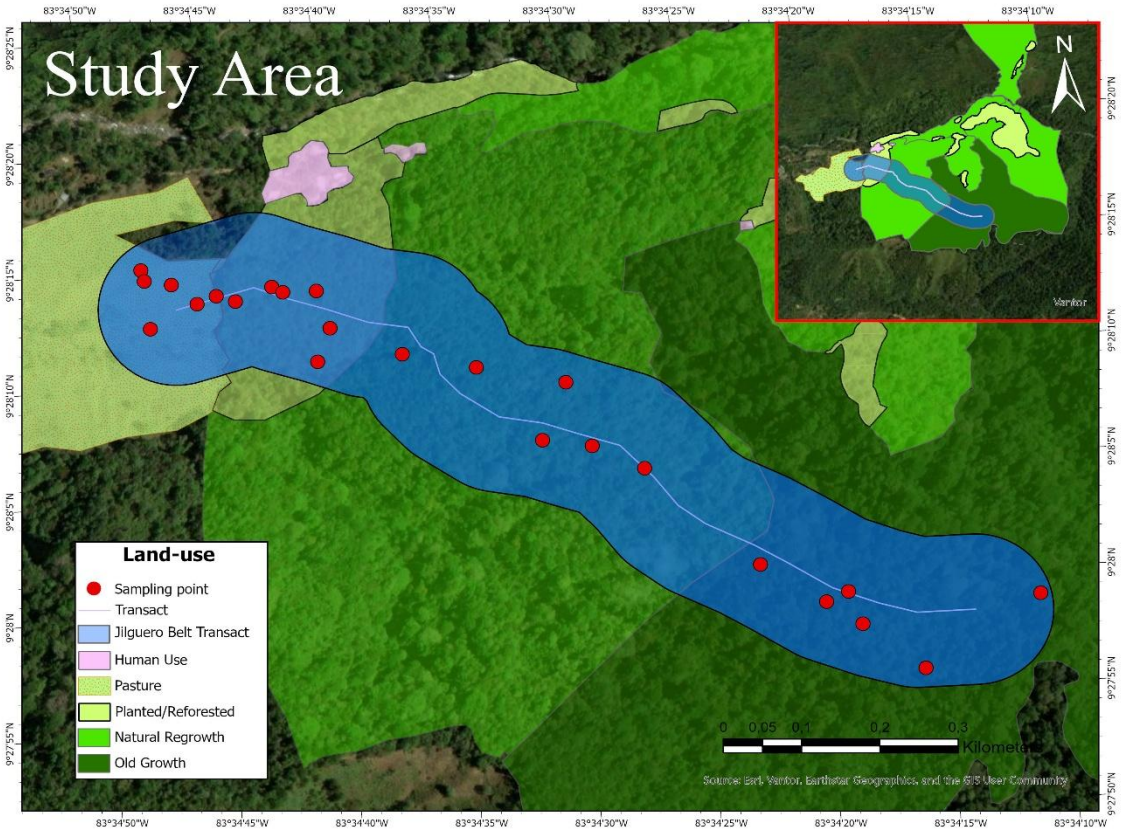

Figure 4: Map of study area with sampling points and the different land-use types along the transect of El Jilguero trail. Source: Author

Restoration gradient and land-use types

The El Jilguero trail within the reserve provides a continuous land-use gradient spanning from pasture to primary old growth forest across a relatively short spatial distance, making it well suited for evaluating restoration. Four land-use types were identified along this gradient, representing different stages of ecosystem condition and together forming a gradient from degraded to intact reference condition against which soil and biological indicators of restoration were evaluated. The four land-use types are described below in order of increasing restoration status.

- Pasture (~1,575-1,600 m) represents the degraded baseline in this study. This plot consists of open, grass vegetation actively used for cattle grazing, with no tree cover. The exposed soil surface and cattle trampling are characteristic of the most degraded condition along the gradient.
- Reforested (~1,600-1,675 m) is a planted forest, actively reforested with native pioneer and climax tree species as part of the reserve's reforestation programme. Together with the natural regrowth plot, it represents an intermediate stage of recovery, characterised by active human intervention through planting and maintenance.
- Natural regrowth (~1,675-1875 m) is a forest that has regenerated passively following agricultural abandonment, without active planting. Species composition here is determined by natural colonisation dynamics rather than management, and it forms the intermediate stage of the gradient alongside the reforested plot.
- Old growth (~1,875-2,000 m) forest represents the least disturbed condition and serves as the ecological reference model. This plot consists of primary montane cloud forest that was not converted during the period of agricultural expansion, characterised by original and well developed vegetation, abundant epiphytes and a well developed organic soil horizon.

The elevation range across the gradient (~425 m) means that the land-use types also differ in altitude. This is an characteristic of the study site layout and is acknowledged as a potential confounding factor in interpreting the results.

Sampling design

A stratified random sampling design was applied within a 200 metre belt transect along the El Jilguero trail. The transect was stratified by land-use type, with six monolith sampling locations established within each of the four land-uses, giving a total of 24 sampling locations. Stratification by land-use rather than a fixed interval sampling was chosen to ensure equal representation of each land-use type for statistical comparison, given that the four land-use types differ considerably in their area along the transect.

Sampling locations were generated in advance using ArcGIS, with 12 candidate points generated per land-use, the first 6 accessible points were used for sampling. If a selected point was inaccessible due to terrain conditions such as steep slopes, dense vegetation, rocks or the presence of a waterbody and no suitable alternative was found within a 10 metre radius, the next point on the list was used instead. Generating multiple candidate points in advance allowed fieldwork to continue uninterrupted, which was important given that fieldwork had to be completed before afternoon rainfall.

At each of the 24 sampling locations, one soil monolith was dug for macrofaunal sampling, and two additional soil cores were collected for laboratory analysis of soil properties, giving a total of 48 soil cores.

Sampling was conducted across 8 days between 6th and 14th of April 2026 (with an exception on Sunday 12th), due to the time consuming sampling of monoliths with hand-sorting. To minimise the influence of diurnal variation in macrofaunal activity and soil conditions, each sampling session was aimed to be completed between 08:00 and 12:00, targeting three monoliths per land-use per sampling session before cloud cover and afternoon rainfall typically set in. Weather data from the San Gerardo de Rivas meteorological station (IMN, 2026)(Located within the pasture plot) indicated that total rainfall during the sampling period was 11.9 mm, with a mean daily temperature of 18.4°C and mean relative humidity of 89.3%, suggesting relatively dry and stable conditions throughout the sampling period. This partially mitigates the potential influence of day-to-day weather variation on macrofaunal activity and soil moisture readings, though some variation between sampling days cannot be entirely excluded and is acknowledged as a limitation of the study. Figure 5 shows the precipitation rates during the sampling period.

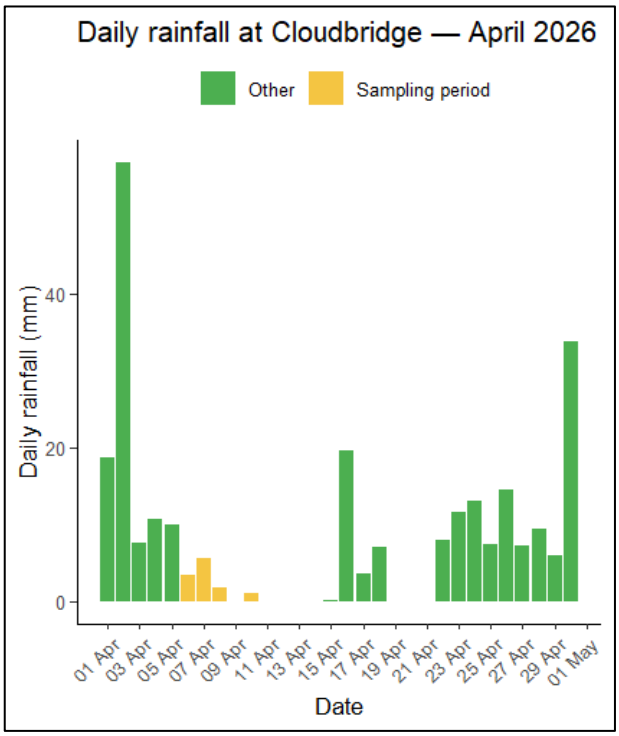

Figure 5: Daily rainfall during the month April 2026, with in yellow the rainfall during the sampling period (6th-14th). Data source: IMN (2026)

Earthworm and Macrofaunal sampling

Soil macrofauna were sampled using the hand-sorting monolith method, following the Tropical Soil Biology and Fertility (TSBF) protocol (Anderson & Ingram, 1993). This method has since been adopted as the ISO standard (ISO 23611-5, 2011) for the characterisation of soil macroinvertebrate communities and has been applied worldwide (Lavelle et al., 2022). At each sampling location, a 25 × 25 cm monolith was excavated to a depth of 30 cm. The excavated soil was hand-sorted, and all macrofauna encountered were collected. Earthworms were separated from other macrofauna, counted and weighed on a portable scale to obtain their biomass. This is done separately since earthworm represent the largest biomass and are considered ecosystem engineers (Jouquet et al., 2006; Keith & Robinson, 2012; Zou & Gonzalez, 1997). All other macrofaunal organisms encountered were weighed together and are categorized as "other macrofauna", since they can also play an important role in regulating ecosystem services (Jouquet et al., 2014). This category included organisms visible to the naked eye such as insect larvae, beetles, ants, centipedes and other invertebrates, though no systematic taxonomic identification was performed. This was chosen because taxonomic identification of tropical soil macrofauna requires specialist expertise beyond the scope of this study and an official permit. Fresh biomass of earthworms and other macrofauna was measured in the field using a portable digital scale. The scale had a resolution of 1 g, meaning that individuals or groups weighing less than 1 g could not be precisely weighed. In such cases, biomass is reported as less than <1 g rather than zero.

Earthworms were photographed individually in the field to allow ecological classification (epigeic, anecic and endogeic). These categories differ in their feeding behaviour, burrowing depth and contributions to soil processes and ecosystem services, as illustrated in Figure 6 (Keith & Robinson, 2012). In standard macrofaunal studies individuals are preserved in ethanol for further identification. However, the goal was to minimise harm to the soil fauna sampled in accordance with principles of Cloudbridge to preserve and conserve. Thus, earthworms and other macrofauna were put back into the field directly after weighing to minimise disturbance as also official permit from the government was needed for extraction and further taxonomic analysis. Classification was done via the photos made in the field and were verified by the WUR supervisor.

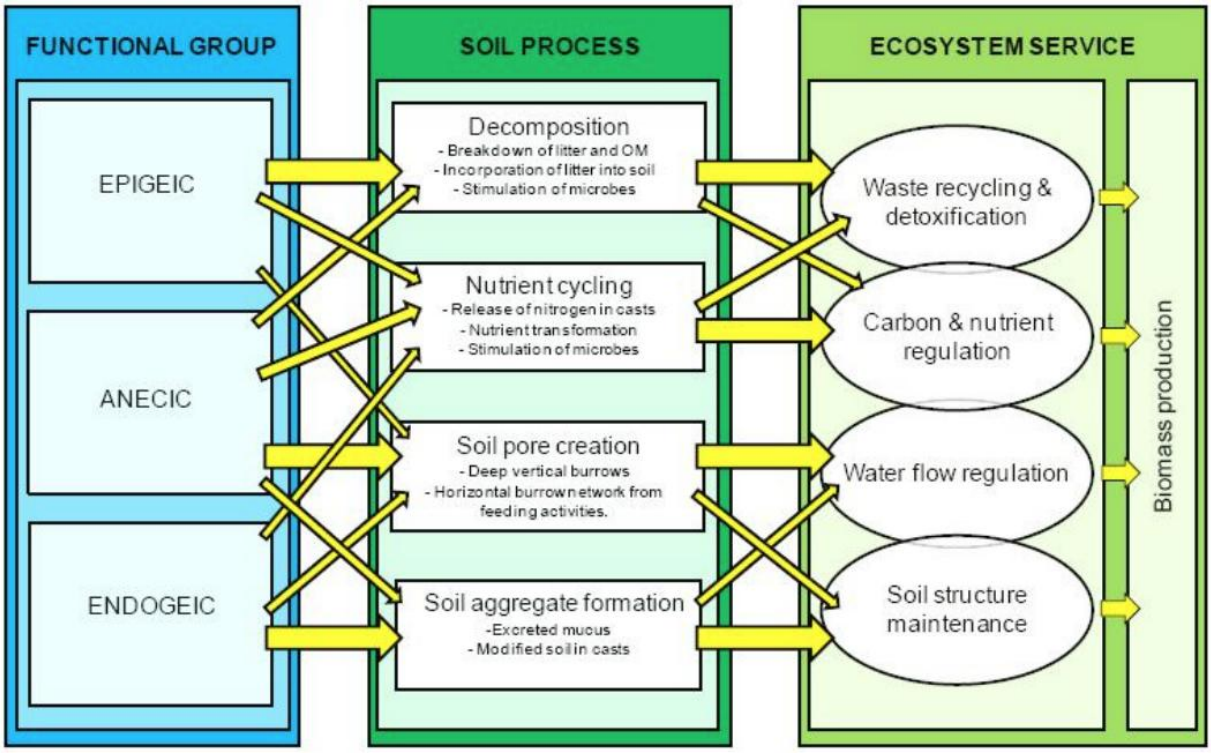

Figure 6: The pathways of broad earthworm functional groups influence on related soil processes that contribute to ecosystem services. Source: Keith & Robinson (2012)

Soil sampling

At each of the 24 sampling locations, two soil samples were collected from the topsoil (0–5 cm depth) using a custom-made steel soil sample ring with a volume of 100 cm^3. The volume of the ring sampler was verified prior to fieldwork by water displacement, confirming the rings volume. Both samples were used for the determination of bulk density and volumetric soil moisture content and the pH measurement. Soil cores were stored in sealed plastic bags and transported to the laboratory for analysis on the same day.

Soil properties determination

Soil moisture content and Bulk density
Soil cores were weighed in the field to obtain the wet mass and immediately upon arrival samples were oven dried until constant mass was reached. Due to equipment limitations, the oven temperature fluctuated above the standard 105°C, averaging approximately 115°C. Further constrained by shared access to the facility's oven, which limited the allowed duration use. To account for this, a drying protocol was applied: samples were dried for an initial period of 2,5 hours, after which they were weighed. Samples then returned to the oven for additional 30 minute intervals, with weighing after each interval, until no further mass loss was detected between consecutive measurements. Once constant mass was confirmed, samples were removed from the oven and their dry mass was recorded. All samples were treated consistently using this protocol, ensuring comparability across samples.

Volumetric soil moisture content (θv) (IAEA, 2008) was calculated as followed :

$$\theta v\ (\%) = (Mw / \rho w) / Vs$$

Where Mw is the mass of water lost during drying (wet mass minus dry mass) and ρw is the density of water (1 g cm^{-3}) and Vs is the known volume of the ring sampler (100 cm^3).

Bulk density (BD) (IAEA, 2008) was calculated as:

$BD\ (g\ cm^{-3}) = Md / Vs$

Where Md is the dry mass of the soil.

pH

Soil pH was measured using a colorimetric method with the LaMotte Model 5029 soil test kit (LaMotte Company, n.d.). For each sample, 5 mL of sieved soil was mixed with 10 mL of demineralized water in a test tube and shaken thoroughly to produce a soil suspension. Five drops of Soil Flocculating Reagent were added, after which the suspension was gently mixed and allowed to settle for approximately 15–20 minutes to obtain a clear supernatant. Approximately 1–2 mL of the solution was transferred to a clean test tube, and two drops of the appropriate pH indicator were added. Where the pH range was initially unknown, a wide-range Duplex indicator was first applied to determine the approximate pH, after which the appropriate narrow-range indicator (Bromcresol green or chlorphenol red) was selected to obtain a more precise measurement. The resulting colour was compared against the corresponding LaMotte colour chart, and soil pH was recorded according to the closest matching value on the chart. Photographs were taken of each colorimetric comparison to document the colour match and are provided in Annex B.

Data preparation

Earthworm abundance recorded per monolith (individuals per 0.0625 m^2) was converted to individuals per square metre (ind. m^{-2}) by multiplying by 16, because their commonly used units. Earthworm biomass and other macrofaunal biomass were similarly converted from grams per monolith to grams per square metre (g m^{-2}). For each of the 24 sampling locations, bulk density, volumetric soil moisture content and soil pH were each measured twice. The two measurements per location were averaged prior to statistical analysis, giving a single representative value per sampling location for each soil property. This approach reduces the influence of variability on the results and made for more convenient data analysis. All data were organised in Microsoft excel prior to statistical analysis.

Statistical analysis

All statistical analyses were performed in R version 4.4.1 (R Core Team, 2024) using RStudio. Statistical significance was assessed at $\alpha = 0.05$.

Normality testing

Prior to selecting statistical tests, the distribution of each variable was assessed using the Shapiro–Wilk normality test. This test determines whether a data sample is normally distributed, which in turn determines the appropriate statistical approach. As earthworm abundance, earthworm biomass, other macrofaunal biomass and soil properties were not normally distributed across all land-use types, and given the relatively small sample size of six samples per land-use type, non-parametric statistical methods were applied throughout.

Kruskal–Wallis test

To determine whether significant differences existed among the four land-use types for a given variable, the Kruskal–Wallis rank sum test was used. This non-parametric equivalent of a one-way ANOVA tests whether the distributions of a variable differ significantly across groups, without assuming normality. It was applied to earthworm abundance, earthworm biomass and other macrofaunal biomass (SRQ1) and to bulk density, volumetric soil moisture content and soil pH (SRQ2).

Pairwise Wilcoxon rank-sum test

Where the Kruskal–Wallis test indicated a significant overall difference among land-use types ($p < 0.05$), pairwise Wilcoxon rank-sum tests were performed as a post-hoc analysis to identify which specific pairs of land-use types differed significantly from one another. Unlike the Kruskal–Wallis test, which evaluates differences across all groups simultaneously, the pairwise Wilcoxon test compares differences in medians between each pair of groups. Holm correction was applied to adjust p-values for multiple comparisons and reduce the risk of Type I error.

Spearman rank correlation

To investigate relationships between earthworm abundance and biomass and the measured soil properties (SRQ3), Spearman's rank correlation coefficient (ρ) was used. Spearman's correlation is a non-parametric method that does not assume normally distributed data and is appropriate for detecting relationships between variables. Earthworm abundance and earthworm biomass were each correlated separately with bulk density, volumetric soil moisture content and soil pH. Both the correlation coefficient (ρ) and the corresponding p-value are reported for each variable pair to describe the strength, direction and significance of each relationship. Additionally, to assess whether soil moisture

measurements were influenced by recent precipitation rather than land-use type, and to contextualise the soil moisture results in relation to SRQ2, a Spearman rank correlation was performed between cumulative rainfall in the three days prior to each sampling date and the volumetric soil moisture content of the corresponding monoliths. Daily rainfall data were obtained from the San Gerardo de Rivas meteorological station (IMN, 2026)

Results

Earthworm abundance and biomass

Earthworm abundance and biomass differed significantly among land-use types (Kruskal-Wallis: $x^2 = 10.48$, df = 3, p = 0.015 and $x^2 = 10.97$, df = 3, p = 0.012, respectively). Earthworm abundance was highest in the reforested plots (mean ± SD: 309 ± 323 ind. m^{-2}), followed by pasture (213 ± 263 ind. m^{-2}), old growth (5.33 ± 8.26 ind. m^{-2}) and natural regrowth (0 ± 0 ind. m^{-2})(Figure 7a). Since earthworm abundance and biomass go hand in hand, a logically following pattern was observed for earthworm biomass, with reforested plots showing the highest values (98.7 ± 115 g m^{-2}), followed by pasture (64 ± 89.9 g m^{-2}), old growth (<1 ± 0 g m^{-2}) and natural regrowth (0 ± 0 g m^{-2}), as can also be seen in figure 7b. Despite the significant overall differences detected by the Kruskal-Wallis test, pairwise Wilcoxon tests with Holm correction did not identify any specific pairs of land-use types that differed significantly from one another for either variable. This likely reflects the low statistical power associated with the small sample size (n = 6 per land-use type) and the high variability within land-use types, particularly in reforested and pasture plots. In the reforested plot the highest variability is present as can also be seen in the standard deviation of ± 323 and both the boxplots 7a,b. This is because two of the monolith samples contained no earthworms, whereas the second and third highest amounts were also found in the reforested plot. As can be seen via the outlying point in 2a, the pasture plot accounted for the highest number of earthworms found in one monolith, while also containing two monoliths with no earthworms.

Visual classification of earthworms via field photography, supported by expert verification, indicated that the large majority of individuals were endogeic.

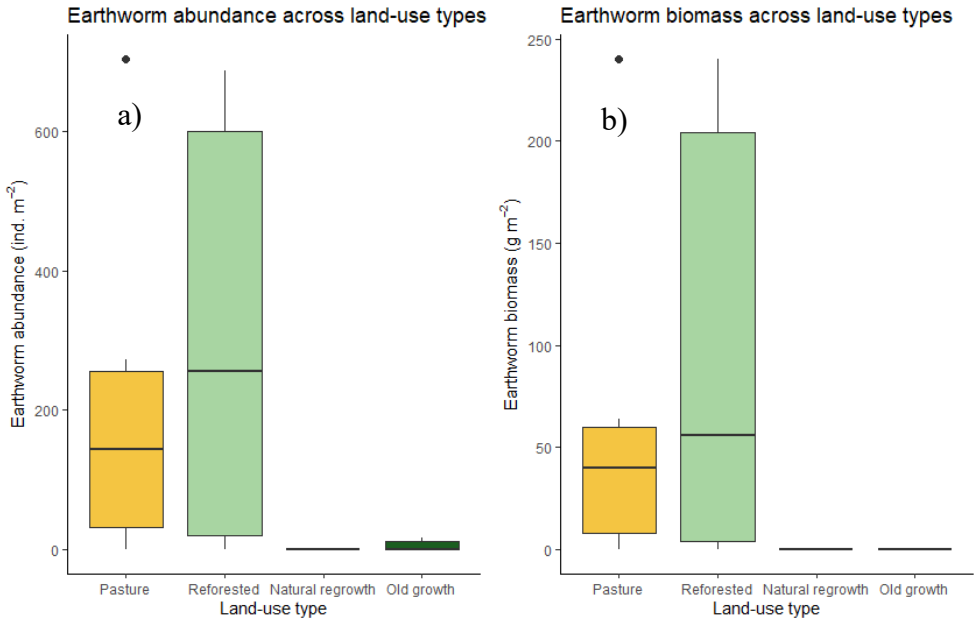

Figure 7: Boxplots of a) total abundance and b) total biomass, across land-use types

Other macrofaunal biomass

Other macrofaunal biomass did not differ significantly among land-use types (Kruskal-Wallis: $x^2 = 3.65$, df = 3, p = 0.301). Mean other macrofauna biomass was highest in old growth (61.3 ± 106 g m^{-2}), followed by pasture (8 ± 8.76 g m^{-2}), reforested (5.33 ± 13.1 g m^{-2}) and natural regrowth (5.33 ± 8.26 g m^{-2}). No pairwise comparisons were conducted given the non-significant overall result. In the old growth one large sized larvae was found, which explains the major difference between the rest of the land-uses, as can be seen in figure 8.

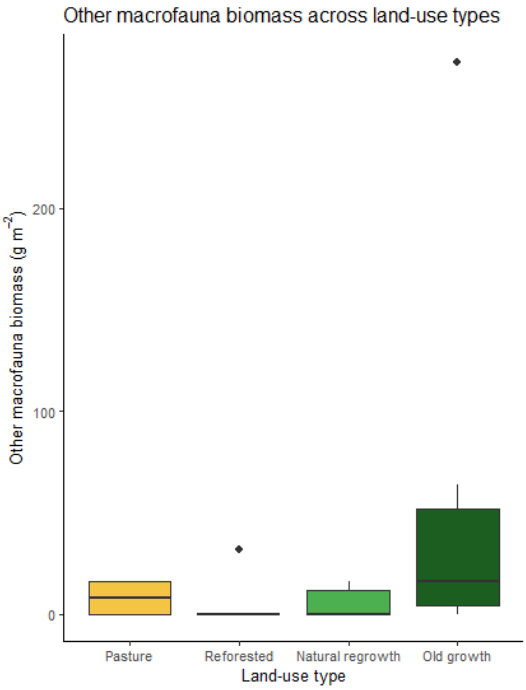

Figure 8: Boxplot of other macrofauna biomass across land-use types

Soil properties

Bulk density differed significantly among land-use types (Kruskal-Wallis: $x^2 = 18.66$, df = 3, p = 0.0003). Pasture had the highest mean bulk density (0.947 ± 0.0585 g cm^{-3}), followed by reforested (0.62 ± 0.0978 g cm^{-3}), old growth (0.399 ± 0.117 g cm^{-3}) and natural regrowth (0.331 ± 0.120 g cm^{-3}). Pairwise Wilcoxon tests are revealed in Table 1, showing that pasture differed significantly from reforested (p = 0.013), natural regrowth (p = 0.013) and old growth (p = 0.013), and reforested differed significantly from both natural regrowth (p = 0.013) and old growth (p = 0.017). Natural regrowth and old growth did not differ significantly from each other (p = 0.423).

Table 1: Pairwise Wilcoxon rank-sum test p-values (Holm-corrected) for bulk density across land-use types.

	Pasture	*Reforested*	*Natural regrowth*
Reforested	0.013*	-	-
Natural regrowth	0.013*	0.013*	-
Old growth	0.013*	0.017*	0.423

*significant at α = 0.05

Volumetric soil moisture content also differed significantly among land-use types (Kruskal-Wallis: $x^2 = 8.31$, df = 3, p = 0.04), though the difference was weaker than for bulk density. Mean moisture content was highest in pasture (0.441 ± 0.0523 cm^3 cm^{-3}), followed by old growth (0.411 ± 0.0673 cm^3 cm^{-3}), reforested (0.384 ± 0.0766 cm^3 cm^{-3}) and natural regrowth (0.316 ± 0.0621 cm^3 cm^{-3}). Pairwise Wilcoxon tests did not identify any specific pairs of land-use types that differed significantly after Holm correction, with the closest comparison being pasture versus natural regrowth (p = 0.052)(Table 2).

Table 2: Pairwise Wilcoxon rank-sum test p-values (Holm-corrected) for volumetric moisture content across land-use types.

	Pasture	*Reforested*	*Natural regrowth*
Reforested	0.595	-	-
Natural regrowth	0.052	0.721	-
Old growth	0.941	0.941	0.184

*No significant pairwise differences detected (α = 0.05)

Soil pH did not differ significantly among land-use types (Kruskal-Wallis: $x^2 = 7.23$, df = 3, p = 0.065). Therefore no pairwise comparisons were conducted. Mean pH ranged from 5.24 ± 0.846 in old growth to 5.90 ± 0.274 in natural regrowth. Although no significant overall difference was detected, old growth plots showed the lowest mean pH (5.24 ± 0.846) and the greatest variability, with individual values ranging considerably across the six sampling locations. The differences between the land-use types in soil properties are illustrated in Figure 9.

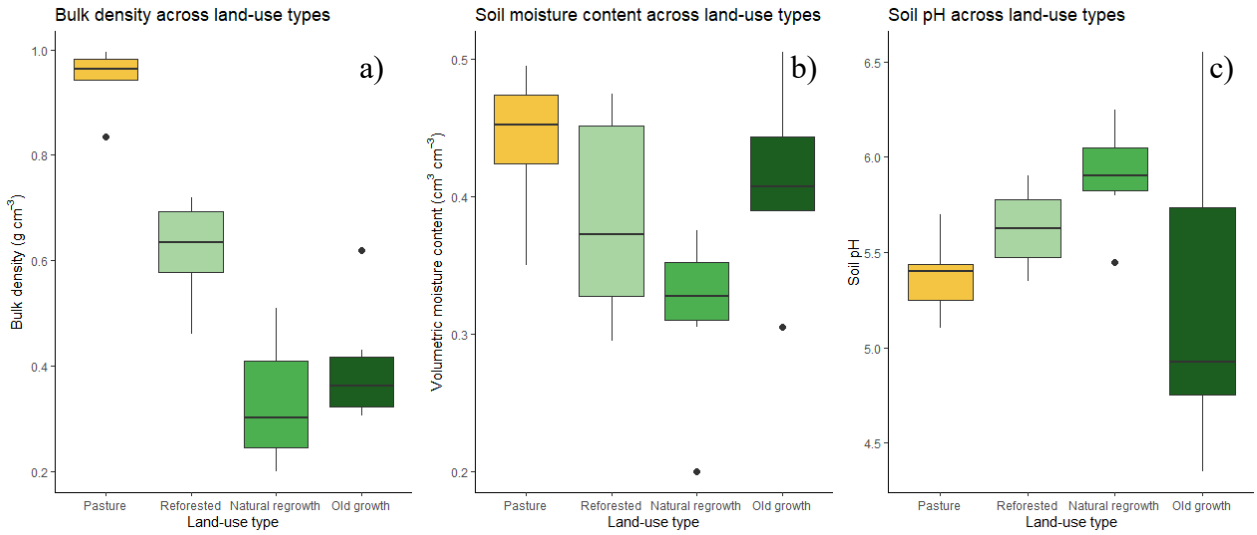

Figure 9: Boxplots of the soil properties with a) bulk density, b) soil moisture content and c) soil pH, across land-use types

Sampling conditions

No significant relationship was found between antecedent rainfall and volumetric soil moisture content (Spearman: ρ = 0.247, p = 0.244; Figure 10), suggesting that variation in soil moisture among sampling locations was not primarily driven by recent precipitation before and during the sampling period.

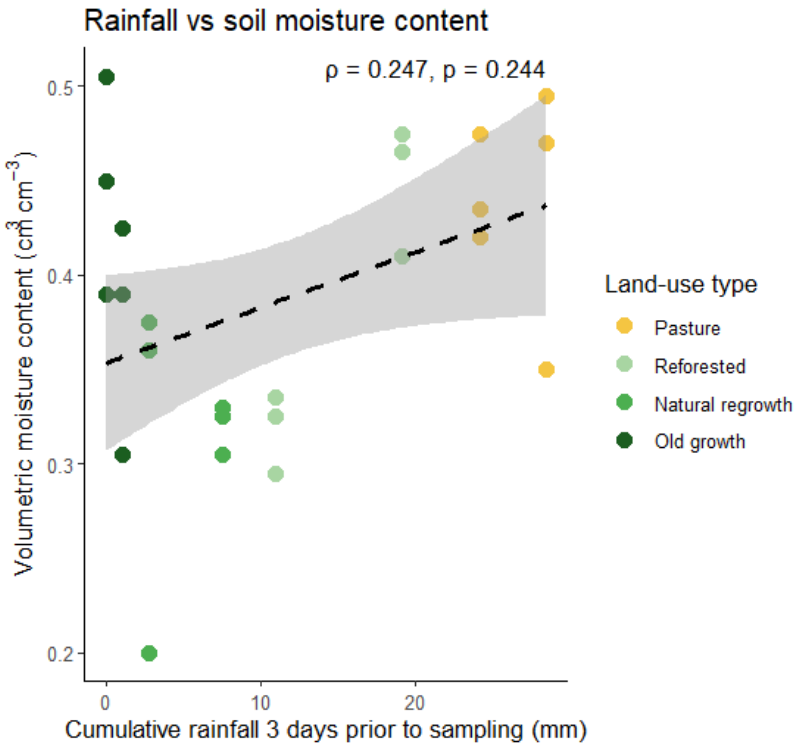

Figure 10: Scatterplot of correlation between cumulative rainfall 3 days prior to sampling and soil moisture content

Relationships between soil properties and earthworm activity

Spearman rank correlations between earthworm abundance and biomass and the three soil properties are summarised in Table 3. Significant positive correlations were found between earthworm abundance and bulk density (ρ = 0.594, p = 0.002; Figure 11a) and between earthworm biomass and bulk density (ρ = 0.566, p = 0.004; Figure 11b), indicating that sites with higher bulk density tended to have higher earthworm abundance and biomass. No significant relationships were found between earthworms and volumetric moisture content or soil pH.

Table 3: Spearman rank correlation coefficients (ρ) and p-values for relationships between earthworm abundance and biomass and soil properties (n = 24)

	Bulk density	*Volumetric moisture*	*Soil pH*
EW abundance (ind. m^{-2})	ρ = 0.594, p = 0.002*	ρ = 0.119, p = 0.581	ρ = −0.352, p = 0.091
EW biomass (g m^{-2})	ρ = 0.566, p = 0.004*	ρ = 0.056, p = 0.795	ρ = −0.145, p = 0.498

*significant at α = 0.05

Although significant positive correlations were found between earthworm abundance and bulk density (ρ = 0.594, p = 0.002) and between earthworm biomass and bulk density (ρ = 0.566, p = 0.004), inspection of the scatter plots (Figure 11) reveals that the data points cluster by land-use type, with pasture and reforested plots occupying the higher bulk density range and natural regrowth and old growth occupying the lower range. Within each land-use type, the relationship between bulk density and earthworm activity is less clear.

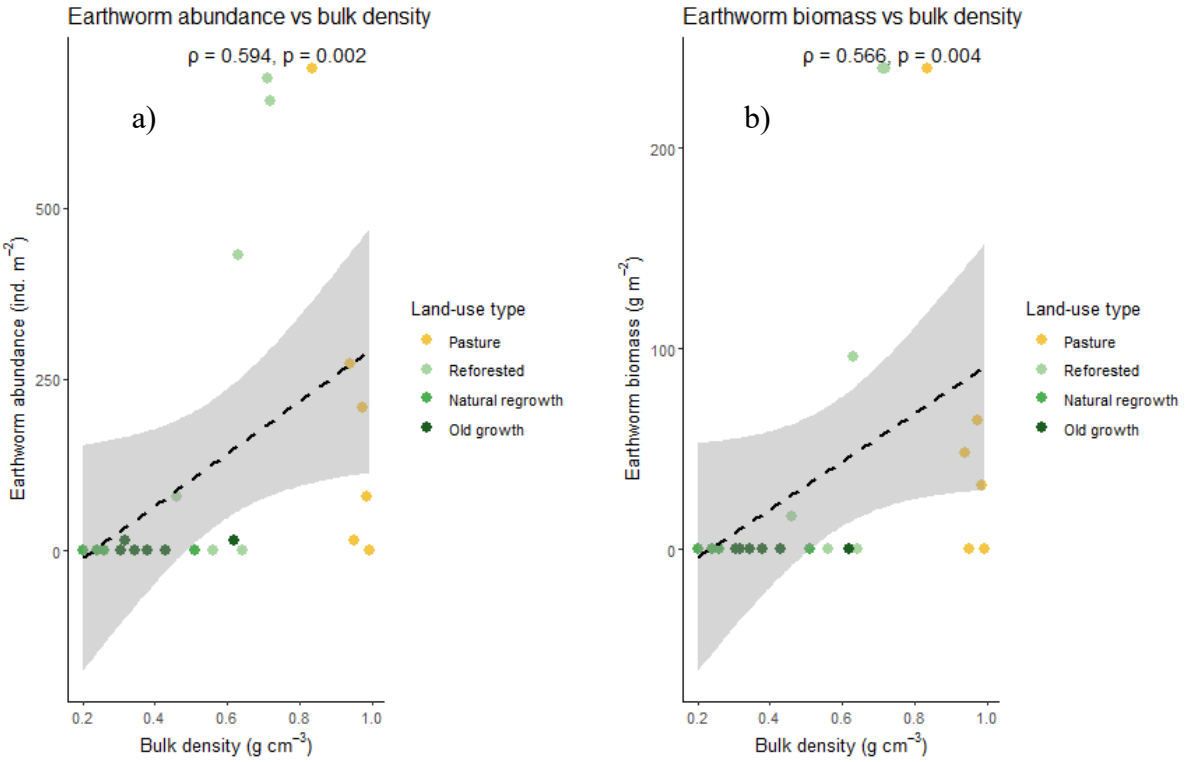

Figure 11: Scatter plots of a) correlation between earthworm abundance and bulk density and b) correlation between earthworm biomass and bulk density

Discussion

Earthworm abundance and biomass across land-use types

Earthworm abundance and biomass were significantly higher in pasture and reforested plots than in natural regrowth and old-growth forest, with natural regrowth no earthworms found. This pattern is the opposite of what you might expect from a restoration perspective, as old-growth forest represented the ecological reference condition and might be expected to support the highest earthworm populations. However, this finding is consistent with broader ecological patterns, namely that grasslands and pastures generally support larger earthworm populations than forest ecosystems, likely due to the better quality and greater abundance of organic resources available in vegetation and a more favourable soil water regime (Lavelle & Spain, 2001). Similar patterns have been reported in tropical restoration studies, where earthworm abundance has been observed to decline during forest restoration relative to pasture (Veldkamp et al., 2020; Zou & Gonzalez, 1997). This suggests that earthworm abundance alone does not reliably reflect restoration progress along a restoration gradient, while they have been proposed as indicators of ecosystem health (Muys & Granval, 1997; Paoletti, 1999) and ecosystem restoration (Dunger & Voigtländer, 2005; Jouquet et al., 2014; Majer et al., 2007; Snyder & Hendrix, 2008).

Interestingly, this pattern contrasts with findings from global assessments of soil macroinvertebrate communities, which report the highest macroinvertebrate abundance in tropical forests (1.895 ± 234 ind. m^{-2}) and savannahs, with progressively lower abundances in pastures (1.178 ± 154 ind. m^{-2}) (Lavelle et al., 2022). However, this represents all macroinvertebrates across tropical forest categories and may not directly reflect the environmental conditions in montane cloud forests. Actually other macrofauna reported the highest biomass in the old growth forest, but this difference was not proven significant. This actually highlights the importance of examining earthworms separately from other macrofauna when evaluating restoration indicators.

Several factors may explain the higher earthworm abundance in pasture and reforested plots. Pasture soils receive grass litter that is relatively easy to decompose and nutrient rich, providing favourable feeding conditions (Curry & Schmidt, 2007; Lavelle et al., 2022). In addition to grass litter quality, cattle dung represents a highly nutritious organic resource that actively attracts earthworms. Studies have shown that earthworm abundance can be up to four times higher directly beneath cattle dung pats compared to surrounding soil (Bacher et al., 2018), suggesting that grazing activity may have directly contributed to the higher earthworm abundance observed in the pasture plots of this study. In contrast, recovering forest soils receive litter that is chemically more complex and slower to decompose, potentially making conditions less suitable for the earthworm species present (Curry & Schmidt, 2007; Zou & Gonzalez, 1997). Soil pH may also play a role, earthworms are generally absent in very acid soils (pH < 3.5) and sparse below pH 4.5 (Räty & Huhta, 2003), though pH values across all land-use types in this study ranged from approximately 4.4 to 6.6, falling within ranges generally considered tolerable for most earthworm species. The relationship between pH and earthworm abundance is discussed further in the section 'Relationships between soil properties and earthworms'.

The near absence of earthworms in natural regrowth and old growth plots may possibly be caused by the higher elevation of these plots (~1,675-2,000) compared to pasture and reforested plots (~1,575-1,675 m) where lower temperatures and litter chemistry could reduce earthworm activity. However, studies of earthworm communities along tropical montane elevation gradients demonstrate that earthworm numbers maintain and could even increase as elevation and rainfall increased and air temperature decreased (González et al., 2007). Fragoso & Lavelle, 1992 also demonstrated that in tropical forest, annual rainfall rather than elevation is the primary driver of earthworm density and biomass, with peak abundance occurring between 2,000 and 4,000 mm annually. Given that Cloudbridge receives approximately 5,130 mm of rainfall per year (Cloudbridge Nature Reserve, 2024a), elevation alone is unlikely to fully explain the absence of earthworms in natural regrowth and old growth plots. Suggesting that elevation alone is unlikely to fully explain the near absence of earthworms in natural regrowth and old growth plots, litter quality and nutrients are therefor more likely the drivers. The large within-group variability observed in reforested and pasture plots is consistent with the spatial distribution of earthworm populations, which may be concentrated in specific areas at the scale of a hectare depending on local variation in soil moisture, organic matter and microhabitat conditions (Lavelle & Spain, 2001).

The observation that the large majority of earthworms is endogeic is consistent with tropical soil studies where endogeic species dominate. Broader ecological pattern observed across climate zones, where epigeic species dominate in cold environments, anecic species in temperate areas, and endogeic species in humid tropical environments (Lavelle & Spain, 2001). This dominance of endogeic species in tropical systems has been confirmed across multiple regions, including Mexico and Central America, where endogeic species dominate both natural and disturbed ecosystems (Fragoso & Lavelle, 1992; Sánchez-del Cid et al., 2024). Endogeic earthworms actively select on mineral soil and organic matter for feeding (Lavelle & Spain, 2001), since quantity and quality of organic matter differs per land-use an abundance pattern is expected..

Other macrofaunal biomass across land-use types

Other macrofaunal biomass did not differ significantly among land-use types ($x^2 = 3.65$, $p = 0.301$), and no clear pattern was observed across the restoration gradient. The elevated mean biomass in old-growth plots (61.3 ± 106 g m^{-2}) was driven by a single large larva found in one monolith, rather than reflecting a consistent community-level pattern. This high within-group variability and the non-significant result suggest that other macrofauna, as measured in this study, do not reliably reflect land-use differences and show limited potential as restoration indicators under the current sampling design.

Variation in soil properties across land-use types

Bulk density

Bulk density showed the most consistent and significant pattern across land-use types, with pasture having the highest values and natural regrowth and old growth the lowest. This is consistent with the well documented effect of cattle grazing on soil compaction, where trampling by livestock reduces microporosity, increases bulk density (Foster, 2001; Muñoz-Villers et al., 2015; Veldkamp et al., 2020) and subsequently the suffering of the infiltration capacities (Bruijnzeel, 2001). The significantly higher bulk density in reforested plots compared to natural regrowth and old growth suggests that active reforestation alone does not reverse the compaction of prior land use, a finding consistent with (Veldkamp et al., 2020) who noted that reforestation reverses many effects of deforestation mainly in the topsoil,

but that soil properties can still deviate from those under natural forest even decades after restoration. The similarity between natural regrowth and old growth in bulk density ($p = 0.423$) is particularly noteworthy, suggesting that passively regenerating forest may recover soil structure to reference conditions, potentially through accumulation of organic matter over time.

Soil moisture

Volumetric soil moisture content differed significantly among land-use types overall, though no specific pairs were significantly different after Holm correction. The absence of a significant relationship between rainfall and soil moisture (Spearman: $\rho = 0.247$, $p = 0.244$) supports the interpretation that the observed variation reflects differences in soil properties and vegetation cover among land-use types rather than short-term weather variation during the sampling period. This is further supported by the dry and stable conditions recorded during sampling (total rainfall 11.9 mm over 9 days;(IMN, 2026)). The higher moisture content in pasture plots relative to natural regrowth is somewhat counterintuitive given the higher bulk density of pasture soils, which would typically be expected to reduce water retention through lower hydraulic conductivity. However, several factors may explain this pattern. First, the lower evapotranspiration of grass cover compared to recovering forest vegetation means that pasture loses less water through transpiration and thus retains more moisture in the soil, consistent with the bucket model described by Foster (2001). Second, the reduced hydraulic conductivity caused by soil compaction may contribute to higher surface moisture retention by slowing the downward drainage of water through the soil. Third, it should be noted that pasture plots were sampled on the 6th and 7th of April, the only days during the sampling period on which rainfall occurred (9.4 mm total), and rainfall had also been recorded in the days immediately prior sampling. Although the Spearman correlation between cumulative prior rainfall (3 days prior to sampling) and soil moisture was not significant across all plots ($\rho = 0.247$, $p = 0.244$), the coincidence of pasture sampling with the wettest days of the period may have contributed to relatively higher moisture readings in those plots specifically, and cannot be entirely excluded as a contributing factor. Though over the whole sampling period of 9 days, the weather was consistent with dry and stable weather conditions (total rainfall 11.9 mm), supporting that soil moisture is reflected by type of land-use rather than varied weather conditions.

Soil pH

Soil pH did not differ significantly among land-use types, which may partly reflect the limited resolution of the colorimetric measurement method used (0.2 pH units), which may not have been sensitive enough to detect slight differences. Though this was also found in the study of Zou & Gonzalez (1997), where pH also didn't differ over successional stages of tropical forest after pasture abandonment. However, the observed pattern, with pasture showing higher mean pH (5.38) than old growth (5.24), is also still consistent with findings from other tropical land-use change studies. Conversion of forest to pastureland typically increases soil pH due to ash input from slash-and-burn clearing, and pastures older than 25 years tend to maintain pH values above those of the original forest soils (Veldkamp et al., 2020). In contrast, natural tropical forests, particularly those on heavily weathered low-activity clay soils, characteristically have low pH values, reported at approximately 4.82 on average across tropical forest soils globally (Veldkamp et al., 2020). The high within-group variability in old-growth pH (5.24 ± 0.846, with individual values ranging from 4.35 to 6.55) likely reflects the complex and heterogeneous litter composition characteristic of established forest ecosystems, where diverse tree species and accumulated organic matter contribute to spatially variable decomposition processes and soil acidification patterns. In contrast, the more uniform pH values in pasture and reforested plots likely reflect the more homogeneous vegetation and litter inputs associated with these land-use types.

Relationships between soil properties and earthworms

Significant positive correlations were found between earthworm abundance and bulk density ($\rho = 0.594$, $p = 0.002$) and between earthworm biomass and bulk density ($\rho = 0.566$, $p = 0.004$). At first, these positive correlations appear to contradict the understanding that soil compaction has a negative effect on earthworm abundance and activity (Jouquet et al., 2014; Söchtig & Larink, 1992), since it slows down their foraging, feeding and mating activities (Binet et al., 1997; Jouquet et al., 2012). However, as visible in the scatter plots (Figure 11), the data points cluster distinctly by land-use type rather than showing a continuous linear relationship across the full gradient. Pasture and reforested plots, which have both the highest bulk density and the highest earthworm abundance, occupy the upper right of the scatter plot, while natural regrowth and old growth, with low bulk density and near-zero earthworm counts, occupy the lower left. This clustering strongly suggests that the observed correlation is driven by land-use type acting as a confounding variable, rather than reflecting a direct causal relationship between compaction and earthworm abundance. In other words, the occurrence of high bulk density and high earthworm abundance in pasture and reforested plots, and at the same time, low bulk density and near-zero earthworm counts in natural regrowth and old-growth forests, suggests that both variables respond to the same underlying land-use conditions. These include vegetation type, quality of organic matter and land-use history. For this study it was assumed that the regenerated land-uses, the reforested and naturally regenerated forest, had the same land-use history, particularly as pasture. However, since they differ so much from each other in both soil properties and earthworm abundance, it raises the question if they actually had the same land-use history. The considerable differences observed between reforested and natural regrowth plots in both bulk density and

earthworm abundance may therefore reflect not only the type of restoration approach applied after abandonment, but also unrecorded variation in prior land-use.

An additional layer of complexity is introduced by the distinction between functionally different endogeic earthworm groups. (Blanchart et al., 2004) identified two functional subgroups of endogeic species with different effects on soil physical properties: "compacting" species, which produce stable macroaggregates and increase bulk density while decreasing water infiltration, and "decompacting" species, which produce labile casts that favour surface sealing but simultaneously increase soil porosity and water infiltration capacity. The net effect of endogeic earthworm communities on soil structure therefore depends on the relative abundance of these two functional types, as well as on soil type and organic matter content (Blanchart et al., 2004; Lavelle & Spain, 2001). Given that the large majority of earthworms in this study were classified as endogeic, and that pasture and reforested plots showed both the highest earthworm abundance and the highest bulk density, it is possible that compacting species are present. However, species identification would be required to confirm this, and this is a limitation of the current study.

No significant correlations were found between earthworm abundance or biomass and volumetric soil moisture content or soil pH. The absence of a significant moisture and earthworm relationship may be caused by the range of moisture values recorded across sampling locations. Earthworms are known to be sensitive to soil moisture, with both very dry and waterlogged conditions reducing activity and abundance (Lavelle & Spain, 2001), but the dry and stable conditions during sampling might be a reason for that. Similarly, the absence of a significant pH and earthworm correlation is consistent with the limited pH variation observed across land-use types and the resolution of the colorimetric pH measurement method used, where it is really easy to make an error. While earthworms are generally absent in very acidic soils below pH 3.5 and sparse below pH 4.5 (Räty & Huhta, 2003), pH values across all plots in this study ranged from approximately 4.4 to 6.6, remaining thus within range of most earthworm species The correlation results suggest that bulk density, soil moisture and pH alone do not explain earthworm distribution patterns across the restoration gradient. But then again, vegetation type, quality and quantity of organic matter and land-use history, lay at hart of the reason behind the distribution pattern.

Indicator potential for restoration assessment

Assessing the progress of ecosystem restoration requires indicators that are sensitive to land-use change, responsive across restoration trajectories, and practically measurable in the field (Webster et al., 2019). The results of this study suggest that the measured variables differ considerably in their suitability as restoration indicators, and that no single indicator alone provides a complete picture of restoration progress.

Bulk density

Of all variables measured, bulk density showed the strongest and most consistent pattern across land-use types, with significant differences between all pairs except natural regrowth and old growth. Pasture had the highest bulk density, consistent with the well-documented compaction effect of cattle trampling and machinery use during agricultural management (Muñoz-Villers et al., 2015; Veldkamp et al., 2020). Reforested plots showed intermediate values, significantly higher than both natural regrowth and old growth, which is a notable finding given that reforested and natural regrowth plots share a broadly similar abandonment date of approximately 2002. Rather than showing comparable bulk density values as parallel intermediate restoration stages, the two plots diverged, with natural regrowth showing bulk density values similar to old growth. This suggests that the type of restoration approach (active planting versus passive regeneration) or differences in prior land-use may have produced differences, even when abandonment dates are similar. Alternatively, the higher bulk density in reforested plots may be due to planting process itself, such as foot traffic during tree planting and maintenance activities. The similarity between natural regrowth and old growth in bulk density is noteworthy, suggesting that passive regeneration may be sufficient to recover soil physical structure to reference conditions within approximately 25 years of abandonment. Bulk density is among the most widely used physical soil indicators in restoration assessments, reported in 34% of reviewed restoration studies (Gatica-Saavedra et al., 2023), and the clear and significant differentiation among most land-use pairs in this study supports its suitability as a restoration indicator in MCF systems.

Macrofauna abundance and biomass

Soil macrofauna have been proposed as valuable indicators of tropical soil health and restoration progress due to their responsiveness to changes in plant cover, organic matter quality and soil management (Webster et al., 2019). Their sensitivity to land-use change and role as ecosystem engineers make them particularly suitable for monitoring restoration trajectories, with their abundance and diversity proposed as indicators of the degree of ecosystem restoration (Dunger & Voigtländer, 2005; Majer et al., 2007; Snyder & Hendrix, 2008) and as bioindicators of ecosystem health more broadly (Muys & Granval, 1997; Paoletti, 1999; Ruiz et al., 2011).

Earthworm abundance and biomass were significantly higher in pasture and reforested plots than in natural regrowth and old-growth forest. a pattern that is actually opposite of what is expected from a restoration perspective and complicates their use as straightforward restoration indicators. The results suggest that earthworm abundance does not follow a simple positive trajectory toward the old growth condition, this consistent with findings from other tropical restoration studies where earthworm populations decline during forest succession relative to pasture (Cole et al., 2020; Zou & Gonzalez, 1997). Webster et al. (2019) confirms that earthworm populations are sensitive indicators of early restoration efforts and useful for restoration monitoring, particularly given their roles in improving soil structure and water holding dynamics. Webster's study suggests that earthworms are useful as indicators of early stage restoration (2 years), so since in this study they're assessed after more than 25 years, usefulness as indicators might not be most suitable.

Other macrofaunal biomass did not differ significantly among land-use types ($x^2 = 3.65$, $p = 0.301$), limiting its indicator potential under the current sampling design. The absence of taxonomic identification means that invertebrate diversity could not be assessed. Invertebrate diversity has been reported as the biological indicator with the highest used indicator toward reference sites across restoration studies (Gatica-Saavedra et al., 2023), suggesting that diversity may haven been more informative than biomass as a restoration indicator.

Implications for ecosystem water regulation

One of the primary motivations for this study was the role of montane cloud forests in regulating water, providing stable baseflow, supporting dry season water availability, preventing flooding and providing soil infiltration capacity (Bruijnzeel, 2001; Foster, 2001). The conversion of cloud forest to pasture disrupts these functions, and the results of this study provide some insight in the implications which this disruption brings and through which restoration of MCF is better understood.

Hydrological soil functions

The significantly higher bulk density observed in pasture plots (0.947 ± 0.059 g cm^{-3}) compared to natural regrowth and old-growth forest (0.331–0.399 g cm^{-3}) has direct implications for the water regulating capacity of the soil. Higher bulk density reduces macroporosity and hydraulic conductivity, limiting the rate at which water can infiltrate into the soil (Blouin et al., 2013). Muñoz-Villers et al. (2015) demonstrated this directly in a Mexican cloud forest study, finding that conversion to pasture reduced saturated hydraulic conductivity by one to two orders of magnitude relative to forest, from approximately 696 mm h^{-1} in cloud forest to only 30 mm h^{-1} in pasture. Even on the permeable volcanic soils, this reduction in infiltration capacity promoted significant increases in overland flow, with pasture producing approximately seven times more surface runoff than forest during major storm events (Muñoz-Villers et al., 2015). These findings are consistent with the bucket model described by Foster (2001), whereby compacted pasture soils have a less permeable bucket, less water enters the soil, more runs off the surface, and thus less is stored for dry season baseflow.

The bulk density results of this study suggest that reforested plots, despite active restoration since approximately 2002, still show quite higher bulk density than natural regrowth and old growth plots. This implies that the hydrological soil functions associated with low bulk density (high infiltration capacity, reduced surface runoff and sustained baseflow) may not yet be fully recovered in actively reforested areas at Cloudbridge, even after more than two decades of restoration. In contrast, natural regrowth plots showed bulk density values comparable to old growth, suggesting that passive regeneration may restore soil physical structure to reference conditions within approximately 25 years of abandonment. This is consistent with findings from a Brazilian Cerrado, where passive forest restoration progressively improved soil infiltration rates, hydraulic conductivity and resistance to surface runoff, with better conditions observed with increasing restoration age (Pereira et al., 2022). Muñoz-Villers et al. (2015) similarly found that 20 years of natural regeneration in a Mexican cloud forest was sufficient to restore the original hydrological functionality of the forest, with secondary forest and mature forest exhibiting very similar annual and seasonal flows.

Earthworm activity and water regulation

The implications of earthworm abundance patterns for water regulation are more complex and depend on the functional composition of the earthworm community. Earthworm burrowing activity generally improves soil macroporosity, air permeability and water infiltration capacity, contributing positively to the water regulating function of the soil ecosystem (Blouin et al., 2013; Lavelle & Spain, 2001). As shown in Figure 6, endogeic species, which dominated in this study, contribute primarily to soil aggregate formation and structure maintenance. They contribute less direct to water flow regulation than anecic species, which do deep burrowing and macropore creation, but nonetheless endogeic species influence water flow regulation directly and indirectly. Stable macroaggregates improve macroporosity and water infiltration capacity, and the maintenance of soil structure reduces surface sealing and erosion , all processes provided by endogeic species that support water regulating function of the soil ecosystem (Blanchart et al., 2004; Blouin et al., 2013). However, as discussed in the relationships section of the discussion, endogeic earthworms can have opposing effects on soil structure depending on their functional subgroup. Compacting endogeic species increase bulk density and reduce infiltration rates, while decompacting species increase macroporosity and promote water infiltration (Blanchart et al., 2004).

The high earthworm abundance observed in pasture and reforested plots, together with the high bulk density of these plots, is therefore consistent with the possibility that compacting endogeic species may dominate in these land-use types. If this is the case, the water regulation function of pasture and reforested soils may be further decreased not only by the compaction of prior land use but also by the functional composition of the current earthworm community. Though species level identification would be required to evaluate if either compacting or decompacting species are present.

Overall implications

Taken together, the results suggest that soil restoration, as reflected by bulk density, is closely linked to the recovery of hydrological soil functions, and that natural regrowth may achieve this recovery more rapidly than active reforestation under the conditions observed at Cloudbridge. The earthworm community, while more abundant in the disturbed plots, does not directly indicate improved water regulation capacity given the complexity of endogeic functional groups and their opposing effects on soil structure. According to Veldkamp et al. (2020), changes associated with deforestation continue for decades after forest clearing, eventually extending to deep subsoils and strongly affecting soil functions including nutrient storage and recycling, carbon storage, erosion resistance, and water storage, drainage and filtration. Although reforestation reverses many of the effects of deforestation, mainly in the topsoil, such restoration can take decades and the resulting soil properties still deviate from those under natural forests (Veldkamp et al., 2020). Research in the Cordillera de Talamanca further indicates that disturbed montane forests require at least 60 to 90 years to recover structurally, and potentially an additional 50 years to fully restore biological diversity (Kappelle, 2016), this suggests that soil and hydrological restoration are long term processes that can not be assessed from a single time point. Nonetheless, the significant differences in bulk density observed across land-use types within a relatively short spatial distance at Cloudbridge suggest that restoration is producing measurable changes in the soil properties that support water regulation, showing gradual recovery of this essential ecosystem service.

Limitations and recommendations

Sampling design and sample size

The most fundamental limitation of this study is the small sample size of six monoliths per land-use type (n = 24 total), which limited statistical power throughout the analyses. This contributed to the highly skewed data and very high standard deviation. Future studies should aim for a larger number of samples per land-use type, ideally 8 to 10 or more, also to improve the ability to detect pairwise differences and reduce the influence of within-group variability on statistical outcomes. A specific limitation relevant to the pasture plots is the potential influence of cattle dung. Bacher et al. (2018) demonstrated that earthworm abundance can be up to four times higher directly beneath dung pats than in surrounding soil, and not accounting for dung pat presence may produce unreliable abundance results. In this study, pasture sampling locations were generated randomly, meaning that some monoliths may have been placed in (old) dung, while others were not (although dung was avoided during sampling for hygiene reasons). This could partly explain the high standard deviation observed in pasture earthworm abundance (213 ± 263 ind. m^{-2}), but at the same time this is also a characteristic of the pasture plot. Future studies could record and account for dung pat presence in sampling designs.

The stratified random sampling design ensured equal representation of each land-use type, but the elevation gradient across the four strata (~330 m) is possibly a confounding factor. Natural regrowth and old growth plots were located at higher elevations than pasture and reforested plots, meaning that observed differences in soil properties and earthworm abundance may partly reflect elevational gradients in temperature, litter chemistry and soil moisture rather than land-use effects alone.

Earthworm hand sorting is a time intensive method, and the involvement of multiple field assistants across different sampling days introduces potential variability in sorting efficiency and consistency. Inexperience with monolith sampling at the start of fieldwork affected the efficiency of early samples and could have led to some errors.

Limited protocols

Bulk density and soil moisture calculations rely on accurate dry mass measurements obtained through oven drying. The standard protocol requires drying at 105°C for 24 hours, but due to equipment limitations and shared access to the facility oven, a stepwise drying protocol was applied at an average temperature of approximately 115°C for an initial period of 2,5 hours, after which samples checked for constant mass at 30 minute intervals. While all samples were treated consistently using this protocol, the higher temperature may have caused additional loss of volatile organic compounds, potentially causing a slight overestimation of water loss and therefore of soil moisture content and underestimation of bulk density. Future studies should use a oven with temperature control set precisely at 105°C and for the full 24 hour drying.

Soil pH was measured using the LaMotte Model 5029 soil kit (LaMotte Company, n.d.), which relies on visual colour comparison by the operator and has a measurement resolution of 0.2 pH units. This resolution is coarser than that

achievable with a calibrated electronic pH meter, and the colour comparison introduces subjectivity through human judgement. The non-significant result for pH across land-use types ($p = 0.065$) may partly reflect this methodological limitation rather than a genuine absence of pH differences. Future studies should use a calibrated electronic pH meter to allow more sensitive detection of pH gradients across restoration stages.

Ecological classification of earthworms was based on field photography rather than taxonomic identification, and not all individuals were verified by the supervising expert. The finding that the large majority were endogeic should be interpreted with this limitation in mind. Species identification, which would allow distinction between compacting and decompacting endogeic species, was beyond the scope of this study but would have improved the understanding of the relationships between earthworm communities, soil physical properties and water regulation.

Conclusion

This study evaluated earthworm abundance and biomass, other macrofaunal biomass, and three soil properties as indicators of ecosystem restoration across four land-use types, pasture, reforested forest, natural regrowth and old growth forest at Cloudbridge Nature Reserve, Costa Rica.

Earthworm abundance and biomass differed significantly across land-use types, but maybe not in the direction expected along a restoration gradient. Both were highest in reforested and pasture plots and near zero in natural regrowth and old growth forest, which represents the ecological reference condition. Pairwise comparisons could not identify which specific land-use types differed, reflecting the small sample size and the high within group variability characteristic of earthworm distributions. Other macrofaunal biomass showed no significant differences across land-use types. Visual classification indicated that the large majority of individuals were endogeic, consistent with the dominance of this functional group in tropical soils.

Of the soil properties measured, bulk density showed the clearest and most consistent response across the gradient, decreasing from pasture through reforested forest to natural regrowth and old growth, with significant differences between all land-use pairs except natural regrowth and old growth. Volumetric soil moisture content differed significantly overall but no pairwise differences remained after correction, and soil pH showed no significant differences, partly attributable to the limited resolution of the colorimetric method used. No significant relationship was found between rainfall and soil moisture, indicating that the observed moisture patterns reflect structural differences between land-use types rather than short-term weather variation.

Earthworm abundance and biomass correlated positively with bulk density, but inspection of the data revealed that this relationship is driven by clustering according to land-use type rather than by a direct effect of compaction on earthworms. No relationships were found with soil moisture or pH. Land-use type therefore appears to determine both variables simultaneously, making it difficult to isolate direct soil property effects on earthworm communities within this study design.

Bulk density seems as the most reliable indicator across the restoration gradient, because of its sensitive and consistent reflection of soil physical recovery. Earthworms respond clearly to land-use change and are therefore informative as indicators of land-use transition, but their non-linear response across successional stages limits their use as indicators of restoration progress in montane cloud forest systems. Soil moisture and pH, were lacking to serve as indicators of restoration, due to probably more complex soil process and a higher sampling size might have actually given a different result. These results point toward using multiple indicators together, since bulk density alone does not tell the full story of ecosystem recovery. More precise measurement methods, a larger sample size and measuring more different soil properties may have yielded different results.

Bulk density supports infiltration capacity and hydraulic conductivity, and the recovery of low bulk density values in naturally regenerating plots suggests a corresponding recovery of the infiltration and water storage functions that underpin the water regulating ecosystem service of montane cloud forests. That reforested plots retained significantly higher bulk density than passively regenerating plots of comparable age indicates that active planting does not necessarily accelerate soil physical recovery. Given that structural and biological recovery of disturbed montane forest in the Cordillera de Talamanca is estimated to take well over a century (Kappelle, 2016), the measurable differences detected across a relatively short spatial gradient at Cloudbridge indicate that soil properties as indicators can capture recovery before full ecosystem recovery is achieved.

Use of AI

Artificial intelligence tools were used as supportive instruments for language editing and text structuring. Claude was used to support the statistical analyses conducted in RStudio. As the author had little experience with R or statistical programming in general, Claude assisted with writing and troubleshooting the R code used for data preparation, normality testing, Kruskal-Wallis and pairwise Wilcoxon tests, Spearman correlations and the production of figures. Claude also provided explanations of what the statistical tests do, how to interpret their outputs, and when specific tests were appropriate given the nature and distribution of the data.

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Annex

A. Rainfall data

Monthly rainfall summary, San Gerardo de Rivas meteorological station (IMN, 2026)

Month	Total rainfall (mm)	Mean daily temperature (°C)	Mean relative humidity (%)
Jan/25	42.4	16.9	92.3
Feb/25	186.3	17.0	91.4
Mar/25	73.8	17.8	84.6
Apr/25	404.2	17.8	92.7
May/25	613.3	17.7	95.4
Jun/25	568.8	17.5	95.7
Jul/25	188.4	17.7	93.6
Aug/25	264.0	17.3	94.1

Sept/25	362.3	17.2	94.8
Oct/25	657.9	16.7	96.5
Nov/25	255.6	17.0	96.5
Dec/25	147.8	16.5	94.2
Jan/26	17.8	16.8	89.9
Feb/26	79.2	17.4	89.0
Mar/26	65.3	17.6	85.9
Apr/26	257.8	17.7	91.7
May/26	312.4	18.4	92.1
Jun/26	223.2	17.7	94.3

Daily rainfall during sampling period, San Gerardo de Rivas (IMN, 2026)

Date	Daily rainfall (mm)	Sampling
06/04/2026	3,4	Pasture (P1, P2, P3)
07/04/2026	5,7	Pasture (P4, P5, P7)
08/04/2026	1,8	Reforested (R2, R3, R4)
09/04/2026	0	Reforested (R5, R6, R7)
10/04/2026	1	Natural regrowth (N1, N2, N4)
11/04/2026	0	Natural regrowth (N5, N6, N7)
12/04/2026	0	No sampling (Sunday)
13/04/2026	0	Old growth (O1, O2, O3)
14/04/2026	0	Old growth (O4, O5, O6)

Total rainfall during sampling period: 11.9 mm. Sampling conducted between 08:00 and 12:00 on each sampling day.

B. Soil and monolith data

Soil data

2 soil samples per monolith

Land-use	Soil sample	Moisture content (%)	Bulk density	pH
Pasture	P1.1	0,60	0,96	5,4
Pasture	P1.2	0,39	0,99	5,4
Pasture	P2.1	0,55	0,75	5,4
Pasture	P2.2	0,39	1,13	6,0
Pasture	P3.1	0,38	1,17	5,5
Pasture	P3.2	0,32	0,80	5,4
Pasture	P4.1	0,49	0,96	5,4
Pasture	P4.2	0,35	0,71	5,4
Pasture	P5.1	0,44	0,89	5,3
Pasture	P5.2	0,43	1,01	4,9
Pasture	P7.1	0,52	0,98	5,5
Pasture	P7.2	0,53	0,93	7,0
Avg.if		0,449	0,940	5,550
Avg.		0,449	0,940	5,550

Reforested	**R2.1**	**0,49**	**0,63**	**5,6**
Reforested	R2.2	0,46	0,49	5,5
Reforested	R3.1	0,39	0,70	5,4
Reforested	R3.2	0,43	0,74	5,3
Reforested	R4.1	0,54	0,20	6,2
Reforested	R4.2	0,39	0,72	5,4
Reforested	R5.1	0,28	0,67	5,5
Reforested	R5.2	0,37	0,75	5,4
Reforested	R6.1	0,26	0,58	5,5
Reforested	R6.2	0,33	0,68	5,9
Reforested	R7.1	0,32	0,62	6,1
Reforested	R7.2	0,35	0,66	5,7
Avg.if		0,384	0,62	5,625
Avg.		0,38	0,62	5,63

Natural regrowth	**N1.1**	**0,31**	**0,55**	**5,5**
Natural regrowth	N1.2	0,35	0,31	5,4
Natural regrowth	N2.1	0,30	0,26	6,2
Natural regrowth	N2.2	0,31	0,43	5,6
Natural regrowth	N4.1	0,33	0,31	5,5
Natural regrowth	N4.2	0,32	0,17	6,3
Natural regrowth	N5.1	0,21	0,15	6,2
Natural regrowth	N5.2	0,19	0,25	5,4
Natural regrowth	N6.1	0,38	0,30	6,3
Natural regrowth	N6.2	0,37	0,22	6,2
Natural regrowth	N7.1	0,42	0,54	6,0
Natural regrowth	N7.2	0,30	0,48	6,2
Avg.if		0,316	0,331	5,900
Avg.		0,32	0,33	5,90

Old growth	O1.1	0,32	0,29	4,2
Old growth	O1.2	0,29	0,34	4,5
Old growth	O2.1	0,40	0,35	5
Old growth	O2.2	0,38	0,51	4,9
Old growth	O3.1	0,46	0,35	6,7
Old growth	O3.2	0,39	0,26	6,4
Old growth	O4.1	0,52	0,70	5,4
Old growth	O4.2	0,49	0,54	4,4
Old growth	O5.1	0,42	0,38	5
Old growth	O5.2	0,36	0,31	4,4
Old growth	O6.1	0,54	0,53	5,9
Old growth	O6.2	0,36	0,23	6,1
Avg.if		0,411	0,399	5,242
Avg.		0,41	0,40	5,24

Monolith data with averaged values for the soil properties

Land-use	Monolith	Biomass EW (gr)	#N_EW	Avg. MC	Avg. BD	Avg. pH	Biomass other (gr)
Pasture	P1	4	13	0,50	0,98	5,40	0
Pasture	P2	3	17	0,47	0,94	5,70	1
Pasture	P3	2	5	0,35	0,99	5,45	1
Pasture	P4	15	44	0,42	0,84	5,40	1
Pasture	P5	0	1	0,44	0,95	5,10	0
Pasture	P7	0	0	0,48	1,00	5,20	0
Avg.if		4	13,33	0,441	0,947	5,4	0,5
Avg.		4	13,33	0,441	0,947	5,4	0,5

Land-use	Monolith	Biomass EW (gr)	#N_EW	Avg. MC	Avg. BD	Avg. pH	Biomass other (gr)
Reforested	R2	0	0	0,48	0,56	5,55	0
Reforested	R3	15	41	0,41	0,72	5,35	0
Reforested	R4	1	5	0,47	0,46	5,80	2
Reforested	R5	15	43	0,33	0,71	5,45	0
Reforested	R6	6	27	0,30	0,63	5,70	0
Reforested	R7	0	0	0,34	0,64	5,90	0
Avg.if		6,17	19,33	0,384	0,620	5,6	0,33
Avg.		6,17	19,33	0,384	0,620	5,6	0,33

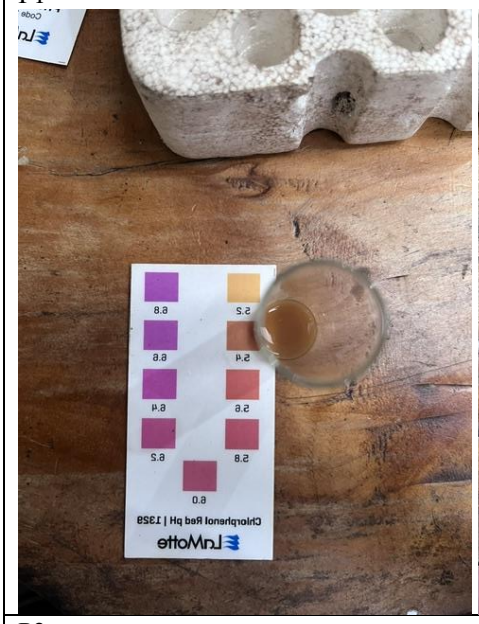
Land-use	Monolith	Biomass EW (gr)	#N_EW	Avg. MC	Avg. BD	Avg. pH	Biomass other (gr)
Natural regrowth	N1	0	0	0,33	0,43	5,45	0
Natural regrowth	N2	0	0	0,31	0,35	5,90	0
Natural regrowth	N4	0	0	0,33	0,24	5,90	1
Natural regrowth	N5	0	0	0,20	0,20	5,80	0
Natural regrowth	N6	0	0	0,38	0,26	6,25	1
Natural regrowth	N7	0	0	0,36	0,51	6,10	0
Avg.if		0,0	0,0	0,316	0,331	5,9	0,333
Avg.		0,0	0,0	0,316	0,331	5,9	0,333

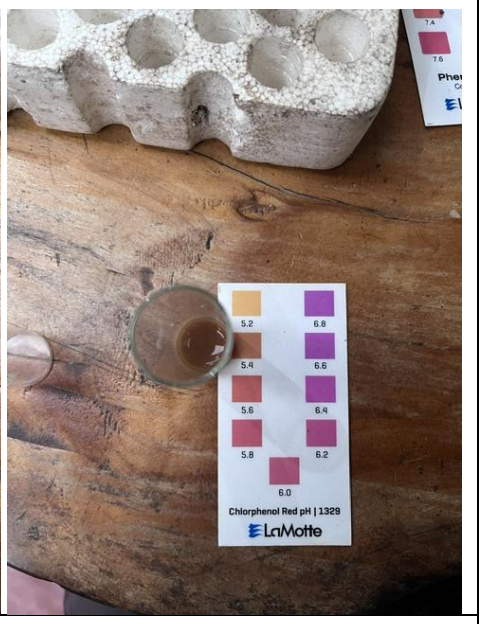
Land-use	Monolith	Biomass EW (gr)	#N_EW	Avg. MC	Avg. BD	pH	Biomass other (gr)
Old growth	O1	0	1	0,31	0,32	4,35	1
Old growth	O2	0	0	0,39	0,43	4,95	17
Old growth	O3	0	0	0,43	0,31	6,55	4
Old growth	O4	0	1	0,51	0,62	4,90	1
Old growth	O5	0	0	0,39	0,35	4,70	0
Old growth	O6	0	0	0,45	0,38	6,00	0
Avg.if		0,0	0,33	0,411	0,399	5,2	3,833
Avg.		0,0	0,33	0,411	0,399	5,2	3,833

Photos of pH determination

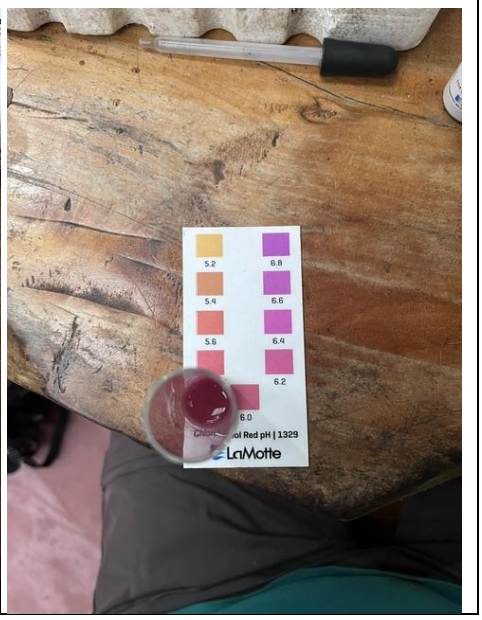
Average was taken per monolith

P1





P2

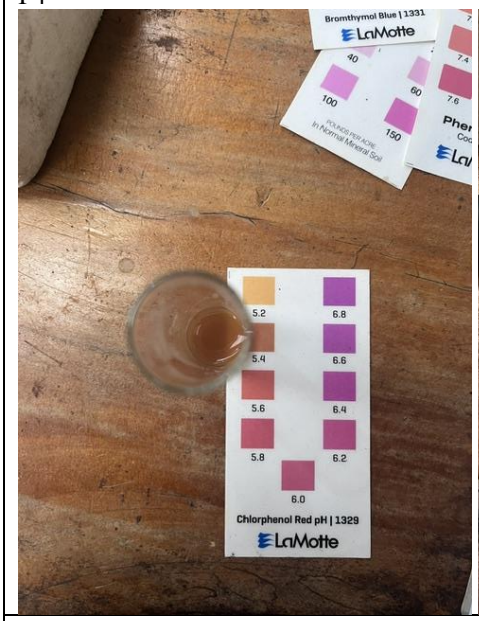


P3


5.2
6.8
5.4
6.6
5.6
6.4
5.8
6.2
6.0
Chlorphenol Red pH | 1329
LaMotte

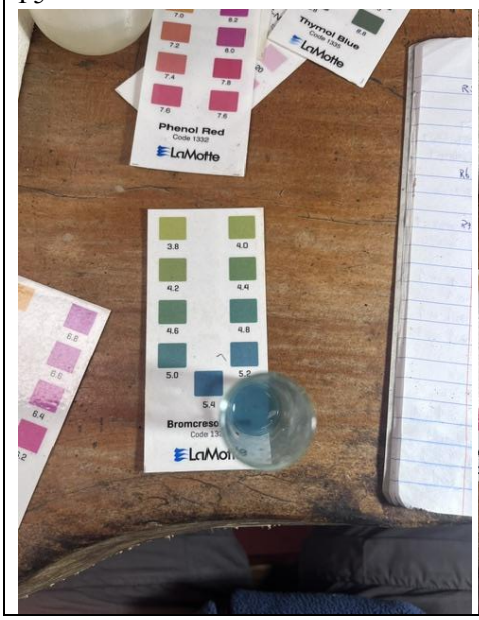

5.2
6.8
5.4
6.6
5.6
6.4
5.8
6.2
6.0
Chlorphenol Red pH | 1329
LaMotte

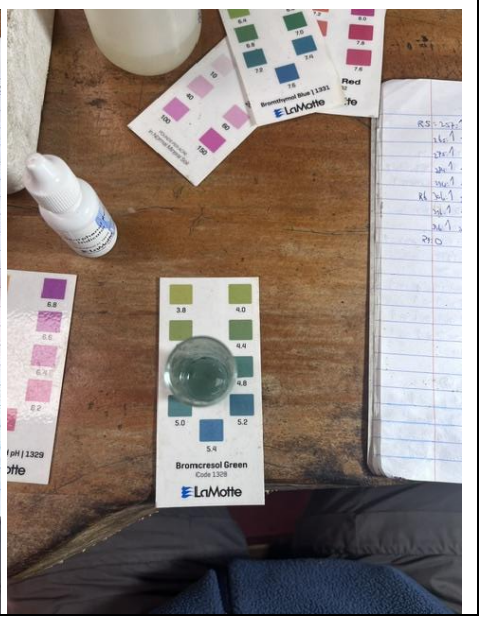
P4


Bromthymol Blue | 1331
LaMotte
40
60
100
150
5.2
6.8
5.4
6.6
5.6
6.4
5.8
6.2
6.0
Chlorphenol Red pH | 1329
LaMotte


Bromthymol Blue | 1331
LaMotte
40
60
100
150
5.2
6.8
5.4
6.6
5.6
6.4
5.8
6.2
6.0
Chlorphenol Red pH | 1329
LaMotte

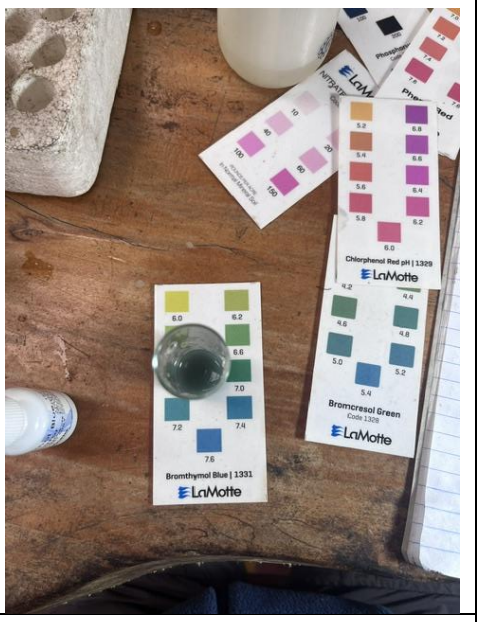
P5


Phenol Red
Code 1332
LaMotte
3.8
4.0
4.2
4.4
4.6
4.8
5.0
5.2
5.4


3.8
4.0
4.2
4.4
4.6
4.8
5.0
5.2
5.4
Bromcresol Green
Code 1328
LaMotte

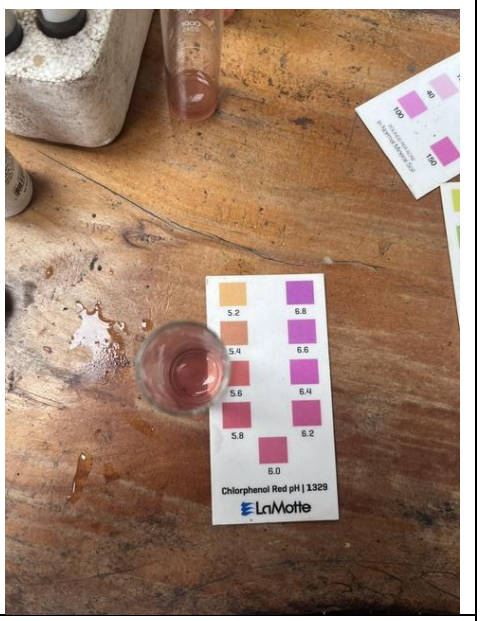
P7


Chlorphenol Red pH | 1329
LaMotte

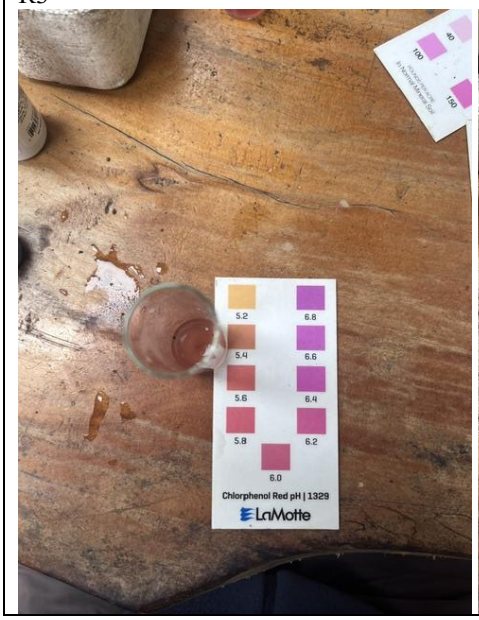

Bromthymol Blue | 1331
LaMotte
Chlorphenol Red pH | 1329
LaMotte
Bromcresol Green
LaMotte

R2


Chlorphenol Red pH | 1329
LaMotte


Chlorphenol Red pH | 1329
LaMotte

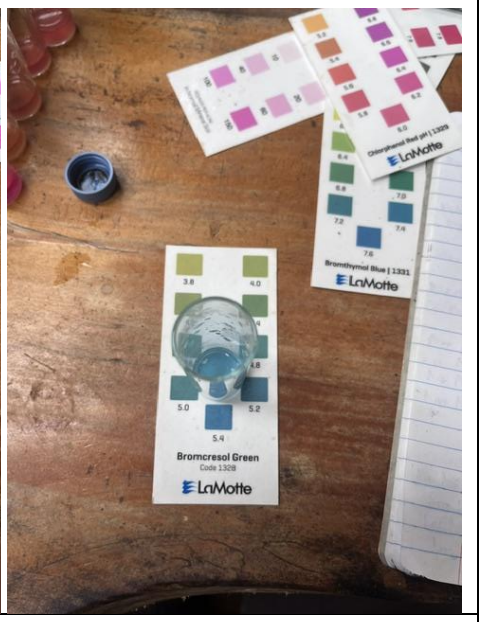
R3


Chlorphenol Red pH | 1329
LaMotte

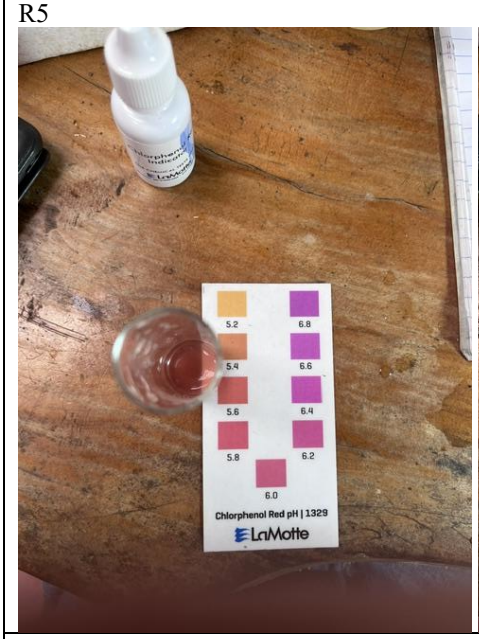

Chlorphenol Red pH | 1329
LaMotte

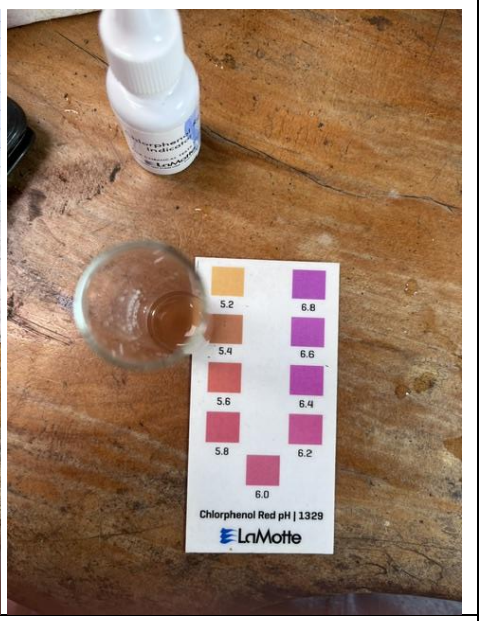
R4


5.2
6.8
5.4
6.6
5.6
6.4
5.8
6.2
6.0
Chlorphenol Red pH | 1329
LaMotte

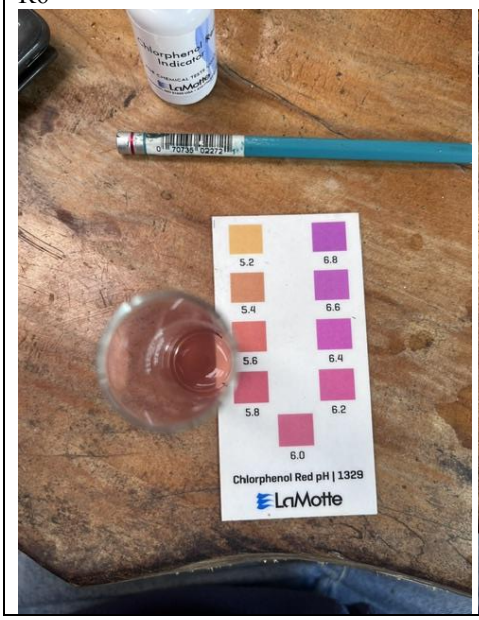

Bromthymol Blue | 1331
LaMotte
Bromcresol Green
Code 1328
LaMotte

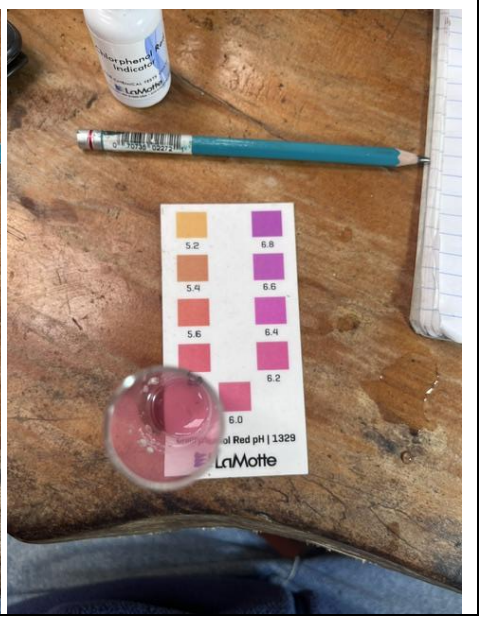
R5


5.2
6.8
5.4
6.6
5.6
6.4
5.8
6.2
6.0
Chlorphenol Red pH | 1329
LaMotte

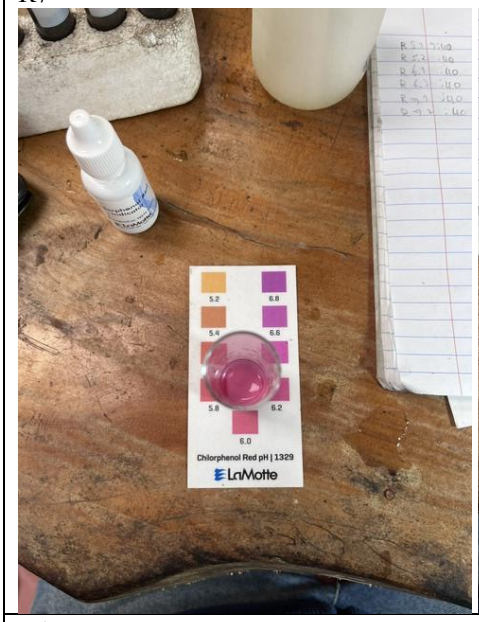

5.2
6.8
5.4
6.6
5.6
6.4
5.8
6.2
6.0
Chlorphenol Red pH | 1329
LaMotte

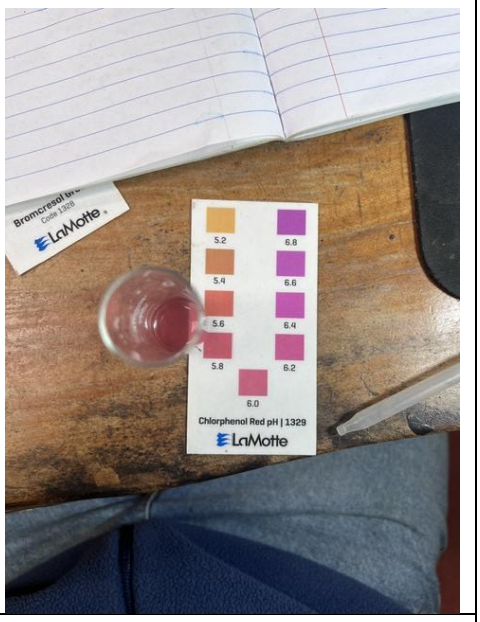
R6


5.2
6.8
5.4
6.6
5.6
6.4
5.8
6.2
6.0
Chlorphenol Red pH | 1329
LaMotte

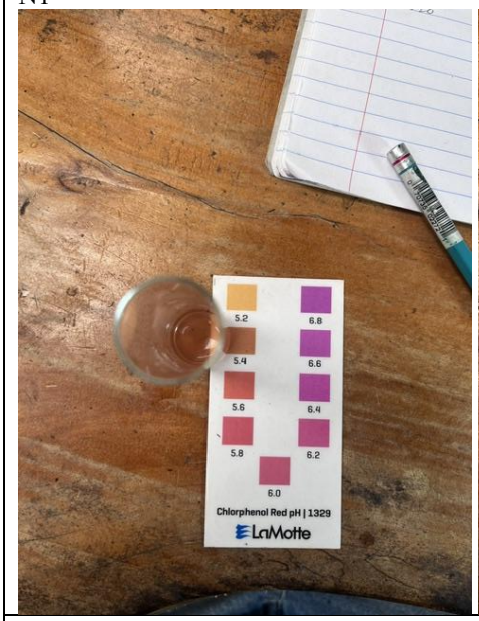

5.2
6.8
5.4
6.6
5.6
6.4
6.2
6.0
LaMotte

R7


5.2
6.8
5.4
6.6
5.6
6.4
5.8
6.2
6.0
Chlorphenol Red pH | 1329
LaMotte

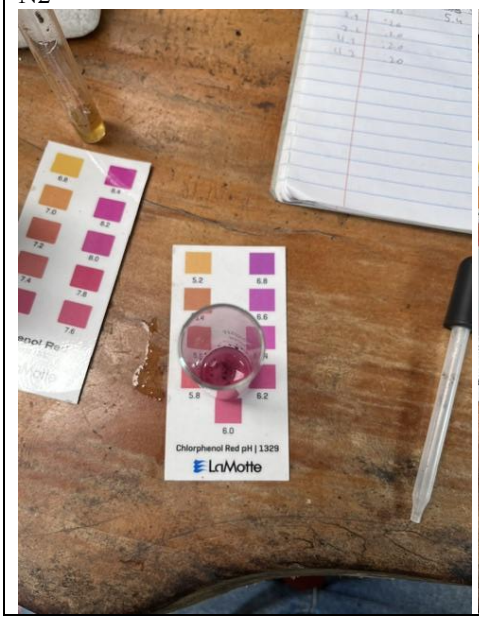

LaMotte
5.2
6.8
5.4
6.6
5.6
6.4
5.8
6.2
6.0
Chlorphenol Red pH | 1329
LaMotte

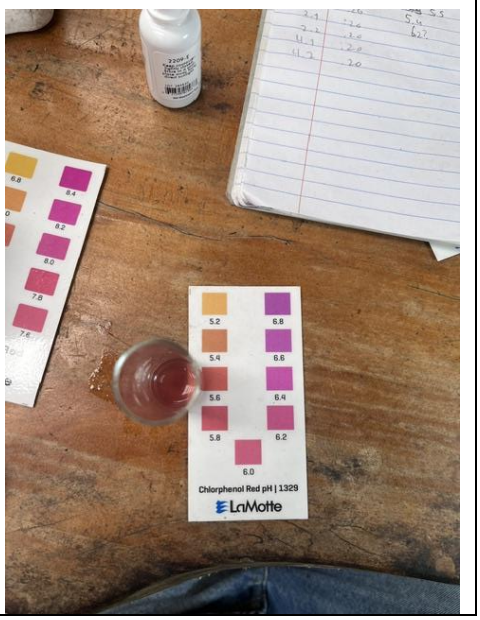
N1


5.2
6.8
5.4
6.6
5.6
6.4
5.8
6.2
6.0
Chlorphenol Red pH | 1329
LaMotte

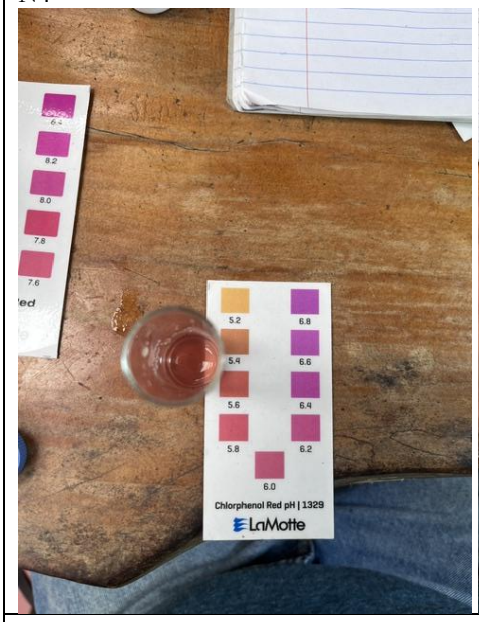

5.2
6.8
5.4
6.6
5.6
6.4
5.8
6.2
6.0
Chlorphenol Red pH | 1329
LaMotte

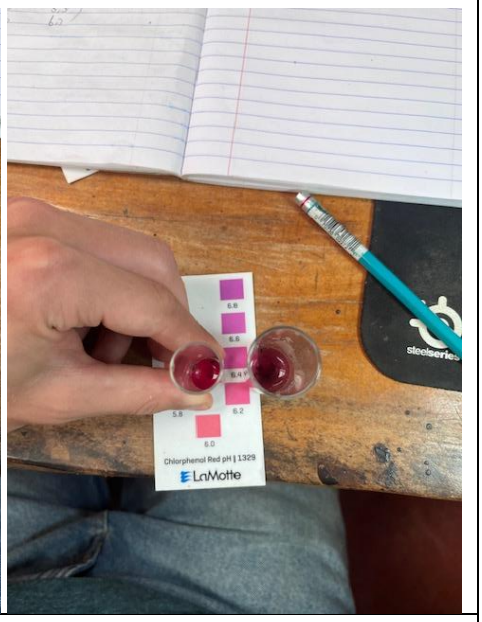
N2


5.2
6.8
5.4
6.6
5.8
6.2
6.0
Chlorphenol Red pH | 1329
LaMotte

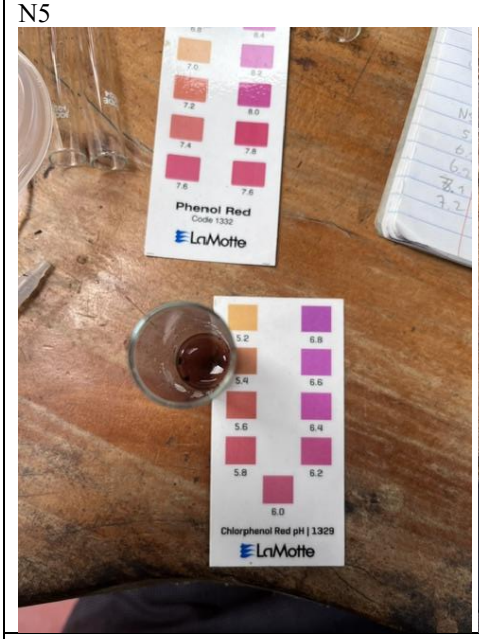

5.2
6.8
5.4
6.6
5.6
6.4
5.8
6.2
6.0
Chlorphenol Red pH | 1329
LaMotte

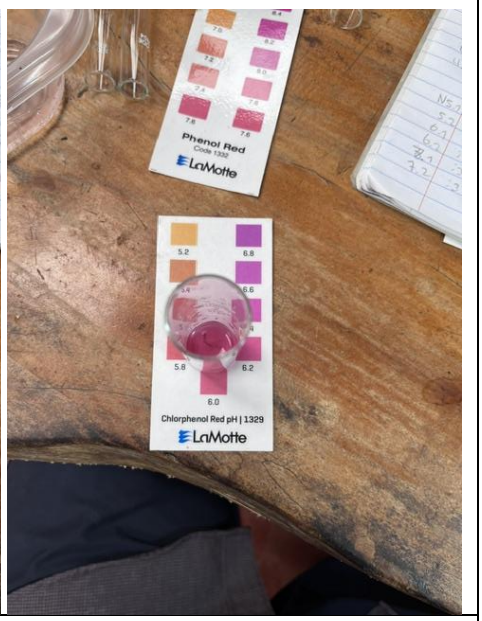
N4


5.2
6.8
5.4
6.6
5.6
6.4
5.8
6.2
6.0
Chlorphenol Red pH | 1329
LaMotte

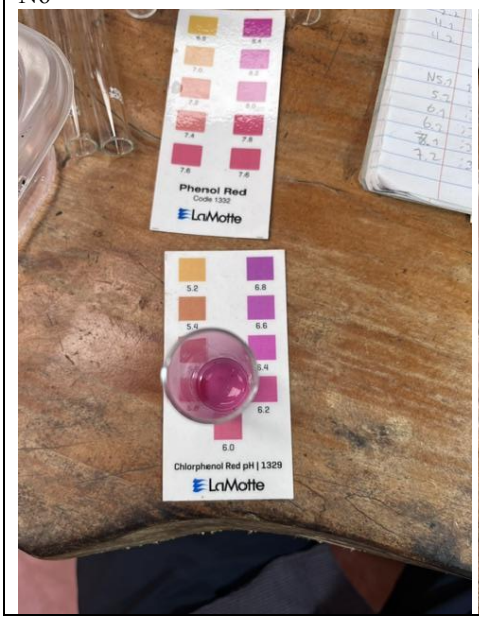

Chlorphenol Red pH | 1329
LaMotte

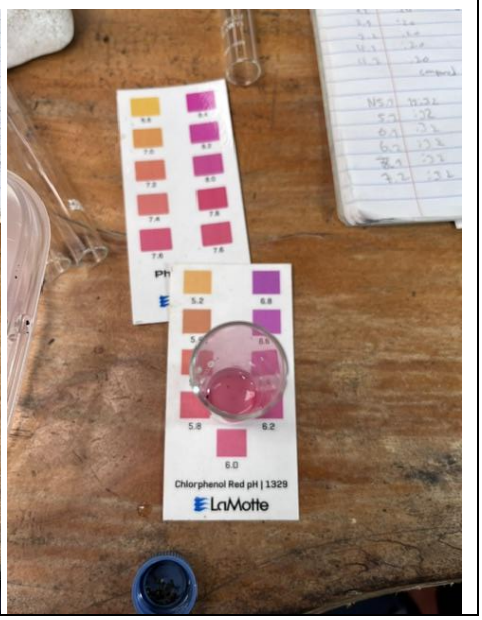
N5


Phenol Red
Code 1332
LaMotte
Chlorphenol Red pH | 1329
LaMotte


Phenol Red
Code 1332
LaMotte
Chlorphenol Red pH | 1329
LaMotte

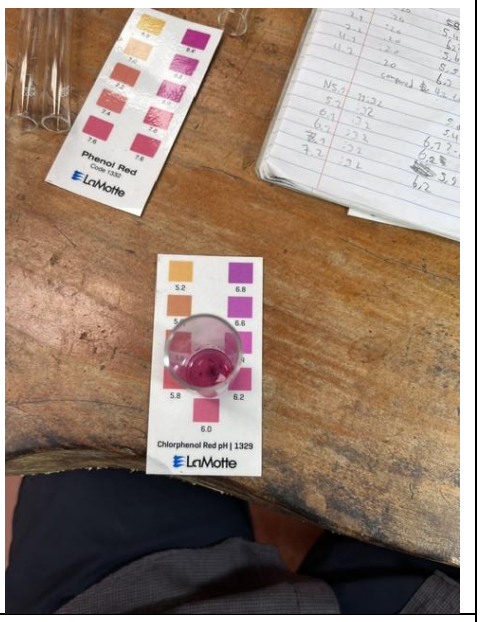
N6


Phenol Red
Code 1332
LaMotte
Chlorphenol Red pH | 1329
LaMotte

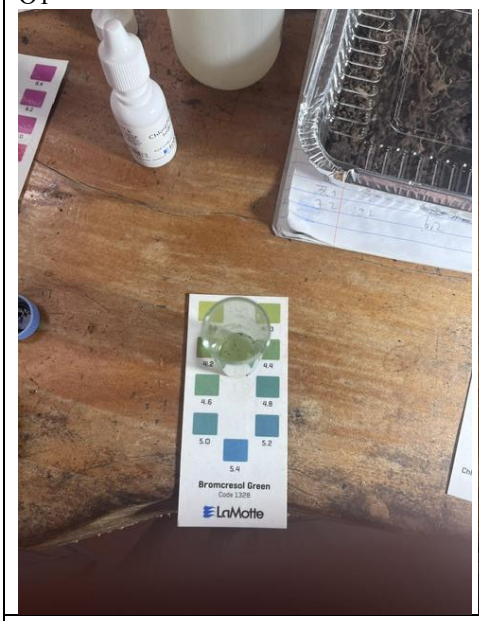

Chlorphenol Red pH | 1329
LaMotte

N7


Phenol Red
LaMotte
Chlorphenol Red pH | 1329
LaMotte


Phenol Red
LaMotte
Chlorphenol Red pH | 1329
LaMotte

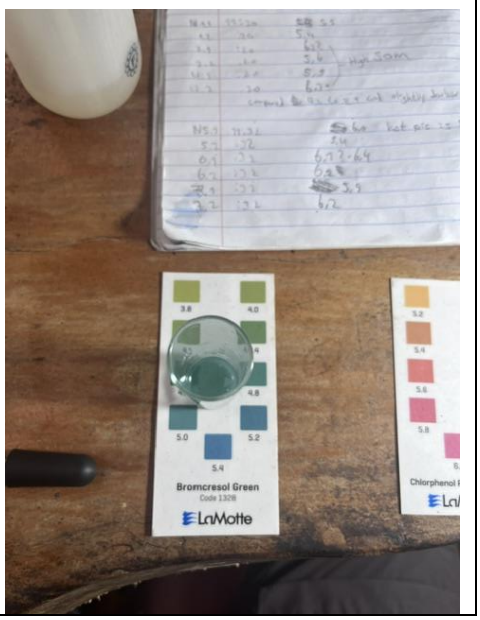
O1


Bromcresol Green
Code 1328
LaMotte

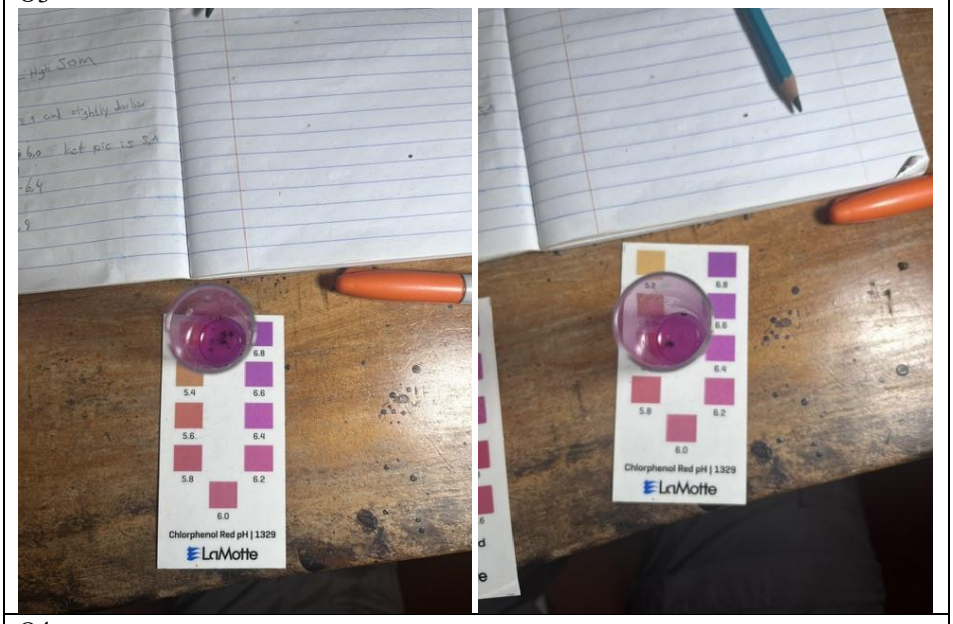

Bromcresol Green
Code 1328
LaMotte

O2

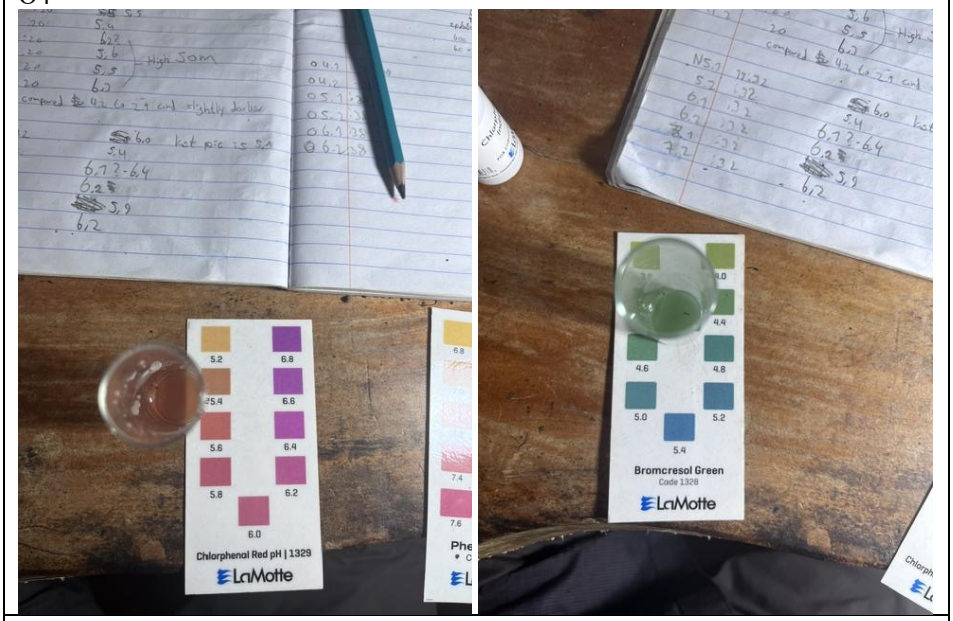

Bromcresol Green
Code 1328
LaMotte


Bromcresol Green
Code 1328
LaMotte

O3


Chlorphenol Red pH | 1329
LaMotte
Chlorphenol Red pH | 1329
LaMotte

O4


Chlorphenol Red pH | 1329
LaMotte
Bromcresol Green
Code 1328
LaMotte

O5


Bromcresol Green
Code 1328
LaMotte
Bromcresol Green
Code 1328
LaMotte

O6


Chlorphenol Red pH | 1329
LaMotte
Bromcresol Green
LaMotte
Phenol Red
LaMotte
Chlorphenol Red pH | 1329
LaMotte
High Sam