**Supplemental Material for: Applied and Environmental Microbiology**

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# Diazotrophs show sign of restoration in Amazon rainforest soils with ecosystem rehabilitation

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**Supplemental material: S1**

**Supplementary Figures:** Figures 1-8

**Supplementary Table:** Table S1&2

**Identification of various diazotrophic groups that responded to change in land-use.** Overall, across all three land use systems, we observed *nifH* sequences from NifH cluster I–V; however, sequences related to clusters I & II were the most dominant (**Fig. S6**). The differences among various land use systems were obvious. NifH–I cluster, dominated by the *Alphaproteobacteria*–related *nifH* gene sequences, showed that the *Proteobacteria*-related diazotrophs are susceptible to changes in land use. *Alphaproteobacteria*–related diazotrophs were abundant in the primary and secondary forests (35–41%), whereas *Gammaproteobacteria* (88%) and *Deltaproteobacteria* (55%) were dominated in the pasture soils. Similar to our results, Dörr *et al*. [1] observed the dominance of methanotrophic *Alphaproteobacteria* in the primary forest soils and *Gammaproteobacteria* species in the farmland created after deforestation of the Amazon. Differences among other, less abundant subgroups across different land use systems were also evident (**Fig. S6**), such as in the NifH–II cluster, *Verrucomicrobium*–related *nifH* gene sequences were retrieved in a relatively high abundance (72%) in the pasture lands as compared to primary and secondary forest sites. Similarly, Ranjan *et al*. [2] observed an increase in abundance of overall *Verrucomicrobium*, based on 16S rRNA gene sequences, in response to the conversion of forest to pasture land. The presence *of nifH* gene and N-fixation by *Verrucomicrobium* spp. have been reported previously [3]. Several of the *nifH* genes OPU (operational protein unit) in the NifH–II cluster lacked any cultured representative that suggest the presence of diazotrophs containing novel lineage of *nifH* genes in these different ecosystems (**Fig. S6**; **Table S1**).

The *nifH* gene has been extensively used as a biomarker gene to assess functional diversity across different environments [4-9], and phylogenetic clustering based on the *nifH* gene has been corroborated with the 16S rRNA gene–based clustering [10, 11]. However, the taxonomic inference drawn based on the *nifH* gene could possibly be influenced by the horizontal transfer of this gene [12]. It is encouraging that some of the diazotrophs related to *Proteobacteria* (Alpha, *Beta*, *Gamma*), *Firmicutes*, and *Actinobacteria* that were detected in this study, have been previously isolated and characterized by 16S rRNA gene sequencing from the primary forest sites [13]. Future ongoing culturing and identification of the diazotrophs across these three different land use systems could further highlight this issue.

## MATERIALS AND METHODS:

**Identification of diazotrophs.** Major diazotrophic groups susceptible to the change in land use were identified by analysis of the representative protein sequences at 88% similarity. Translated *nifH* protein sequences (394,920 sequences, 131,640 per land use system, subsampled based on the land use system with the least number of DNA sequences) were aligned with the *nifH* protein sequences of the reference database from Wang *et al*. [7], and a maximum likelihood (ML) phylogenetic tree was generated using FastTree. Operational protein units (OPUs) were collapsed at 88% amino acid similarity level that was corresponding approximately to the class level differences [14]. Distribution of sequences within each OPU were presented as bar graphs.

**Quantitative PCR for the *nifH* gene.** The abundances of the *nifH* gene sequences across different land use systems were quantified through an absolute quantitative PCR approach. A 25 µl qPCR reaction contained 12.5 µl of iTaq$^{TM}$ Fast SYBR Green Super mix (Bio–Rad Inc., Hercules, CA), 100 nM of the *nifH* primers (same as used for high–throughput sequencing) and 10 ng of the template DNA. The *nifH* gene from *Herbaspirillum seropedicae* (American Type Culture Collection 35892) was cloned into TOP 10 *Escherichia coli* competent cells and used for the generation of standard curves. PCR-grade water without template was used as a negative control that did not show any amplification. The real–time PCR amplification efficiency of *nifH* gene was 98%, and the $R^2$ of 0.99.

Although, we followed the absolute quantification procedure the main purpose was to assess the relative abundance of diazotrophs across three different land-use systems. In general, the extracted DNA samples were relatively pure and the quality of the extracted DNA was very good (as indicated by 260/280 ratios >1.75). Overall, the DNA extraction yield across three land-use systems was very similar (Pasture 25±7.5 ng/µl; Forest 31±3.9; Secondary forest 29±8.5). We did evaluate the presence of potential PCR inhibitors in 15 randomly selected DNA samples (five per land-use system) by comparing the *nifH* gene copies quantified from concentrated and 1:10 diluted DNA samples. We did not observe any differences between concentrated vs diluted samples suggesting the absence of PCR inhibition (data not shown).

**Supplementary Figures:** Figures S1-S8

**Supplementary Figures:**

**Fig. S1:** Taxonomic (black) and phylogenetic (grey) $\alpha$-diversity of the *nifH* genes in response to land use change in the Amazon. Taxonomic diversity was calculated using: A) Margalef species richness index and B) Shannon diversity index. Phylogenetic diversity was calculated as Faith's phylogenetic diversity (Faith, 1992). Overall, 135 000 *nifH* gene sequences (45,000 per land use and 1,500 per sample) were used to calculate $\alpha$-diversity. Error bars represent 95% CI.

**Fig. S2 A-C:** Diazotrophic community turnover in space, or β-diversity at different DNA identity levels: A) 95% DNA identity, B) 90% DNA identity, C) 97% protein similarity (amino acid based) in response to land use change in the Amazon. Differences in the slopes across different land use systems were compared by power model. Upper panel represent the variations in the taxonomic β-diversity and lower panel represent the phylogenetic β-diversity across different land use systems. β-diversity was also calculated from subsampled data i.e., total 135,000 *nifH* gene sequences (45,000 per land use and 1,500 per sample).

**Fig. S3:** Nonmetric multidimensional scaling representation of the *nifH* gene sequence composition at 97% DNA identity for three different land use systems in the Amazon: Subsampled data set containing total 135,000 *nifH* gene sequences (45,000 per land use and 1,500 per sample) were for this comparison.

**Fig. S4:** Nonmetric multidimensional scaling representation of the *nifH* gene sequence composition at different DNA identity (95%, 90%) and protein (97%, 95%) similarity levels for three different land use systems in the Amazon: Primary forest samples (black triangle); Pasture samples (white circles); Secondary forest samples (gray squares). Complete data set containing total 443,600 *nifH* gene sequences and distribution of number of sequences retrieved from different treatment were: primary forest samples (158 261); pasture samples (143,700); secondary forest samples (131,640).

**Fig. S5:** Log-scale abundance of *nifH* gene copies (g$^{-1}$ dry soil) for the three different land use systems. Each bar represents about 90 samples (30 biological replicates per site and 3 technical replicates) and the error bar represents 95% CI.

**Fig. S6:** Maximum likelihood phylogenetic tree based on translated amino acid sequences of the *nifH* gene from three different land use systems in the Amazon rainforest. The size of eachcircler is proportionate to the log number of sequences within an OPU. Total number of sequences in an OPU are also shown next to the taxon names. Overall, a total of 394,920 *nifH* gene sequences were used and the comparison was normalized to 131,640 sequences from each treatment based on the least number of sequences retrieved from secondary forests (131,640 *nifH* gene sequences were randomly sampled from primary forest and pasture samples to match equal numbers). Within an operational protein unit (OPU) translated amino acid *nifH* gene sequences were collapsed at 88% similarity. Numbers at the nodes reflect bootstrap support values above 70% with branches within clusters being collapsed. Operational protein units (OPUs) and the closest matched sequences within each OPU that were obtained through the *nifH* gene reference database were reported in (**SI Table 1**).

**Fig. S7: A).** Operational taxonomic unit (OTU) accumulation curve for three land use systems in the Amazon. Total 443,600 *nifH* gene sequences retrieved from different treatments (primary forest samples (158,261); pasture samples (143 700); secondary forest samples (131,640) were used to generate accumulation curve. The observed number of OTUs were calculated at 97% DNA similarity.

**B).** The accumulation curve for each sampling location. Total nine sites, three per land use system (Forest FI, II, III; Pasture P72I, II, III, and Secondary forest SCI, 93, 98).

**Fig. S8:** A spatially explicit sampling scheme that indicates the different sampling locations within three land use systems. A square box represents 12 sampling points at each location. Overall 36 soil samples per land use system.

**Supplemental Table:**

**Table S1:** Operational protein units (OPUs) and the closest matched sequences within each OPU that were obtained through the *nifH* gene reference database by Wang et al., (2013).**Table S2:** Soil physiochemical characteristics of thee land-use systems in the Amazon.

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Fig. S1

# 97% DNA identity Alpha diversity

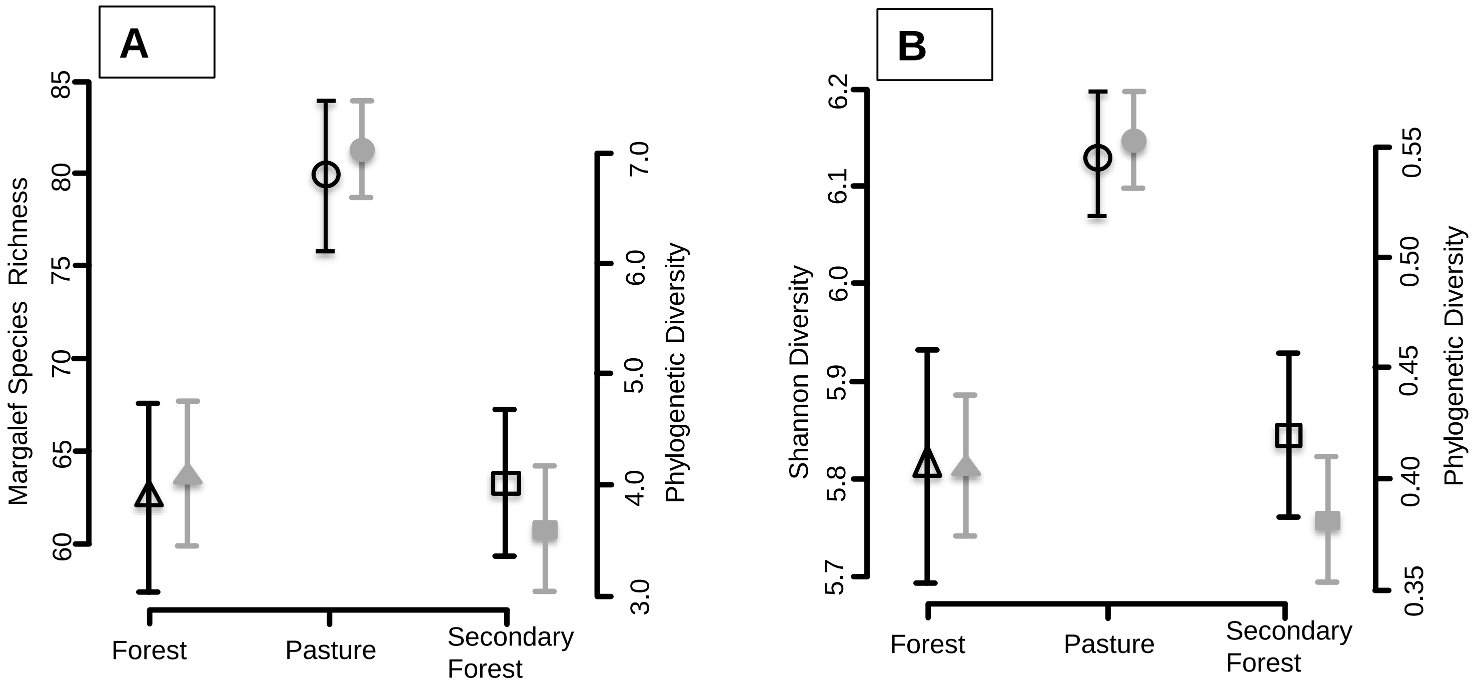


Fig. S2A

## 95% DNA Bray-Curtis and unifrac metrics

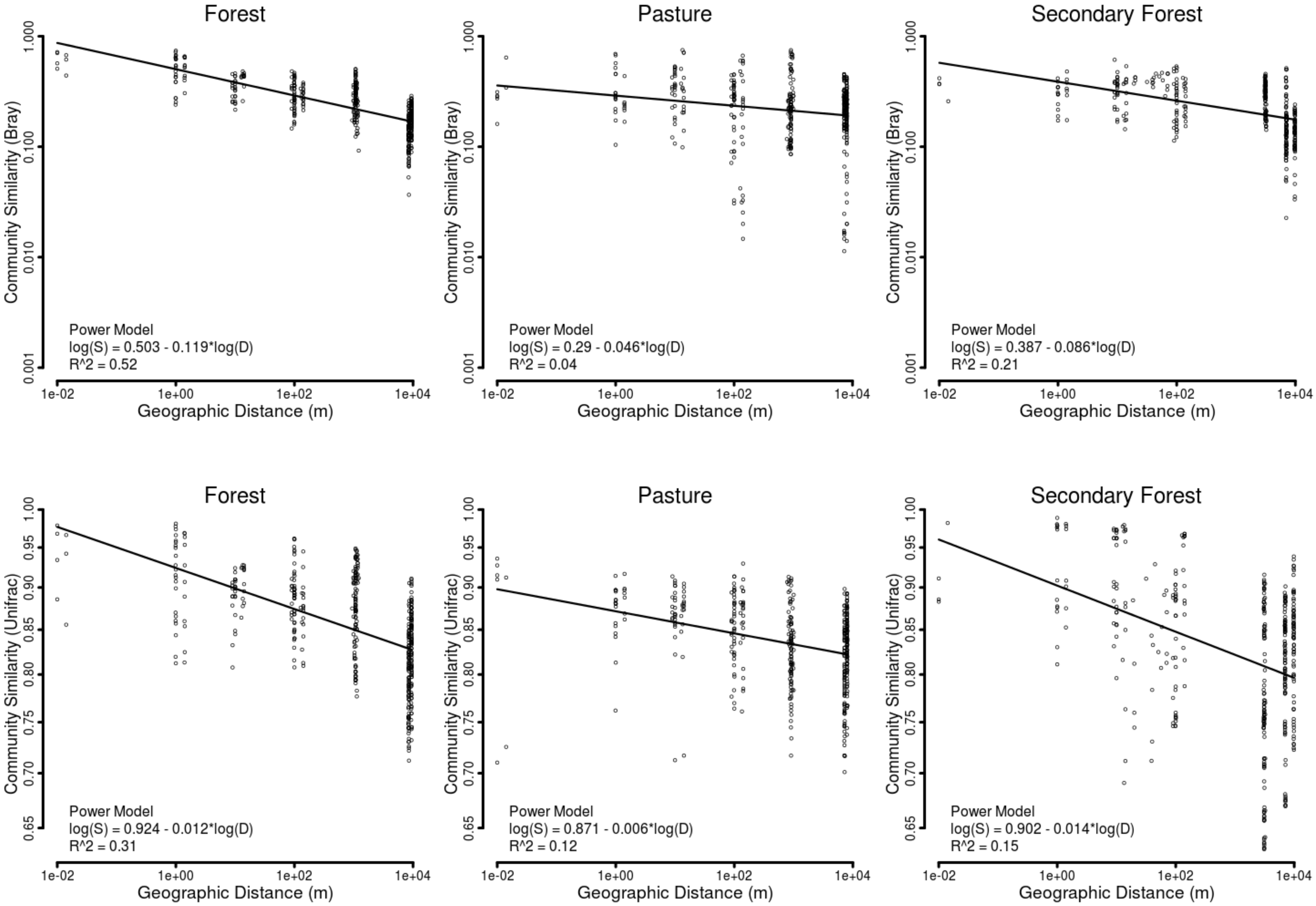


Fig. S2B

# 90% DNA Bray-Curtis and unifrac metrics

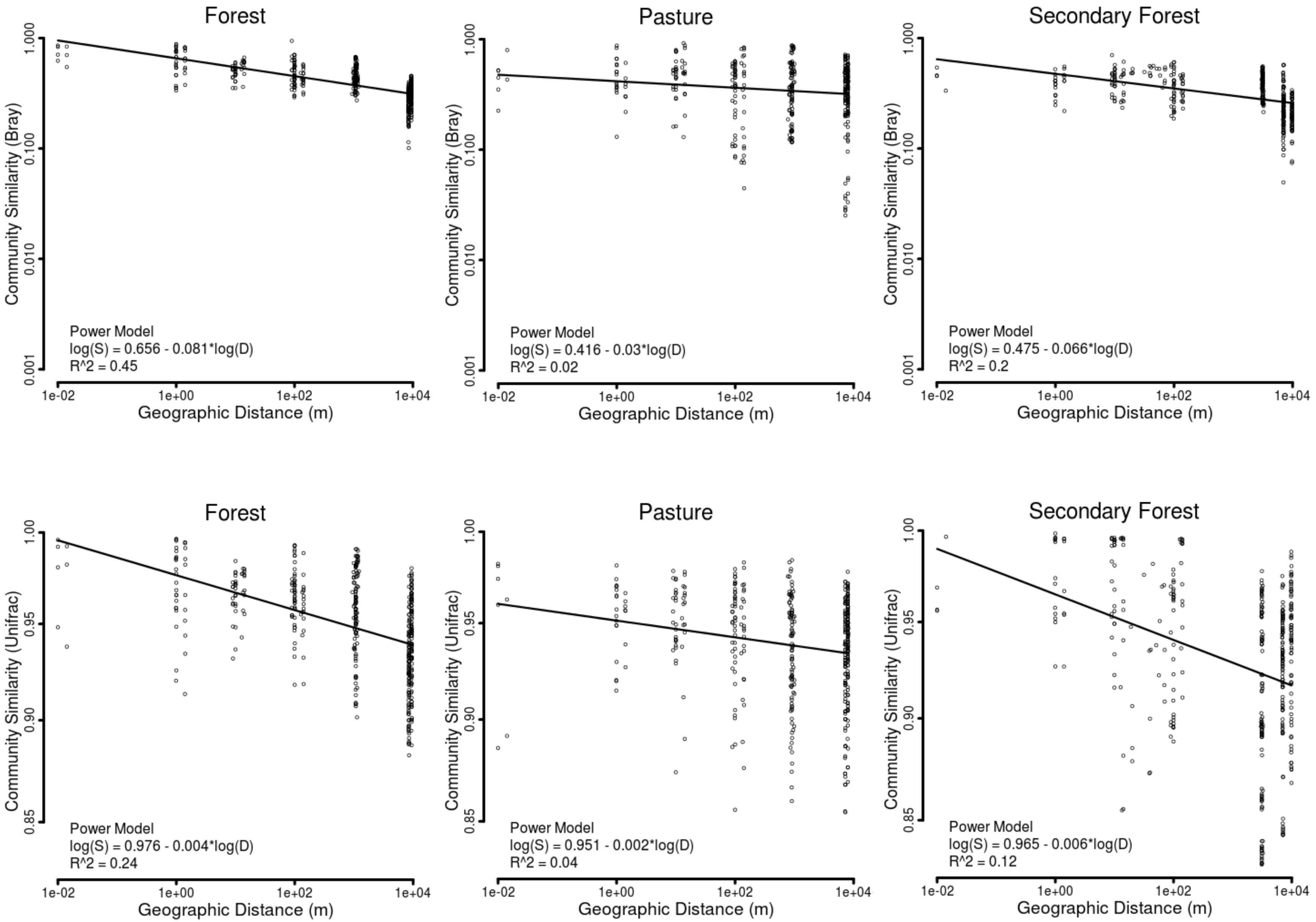
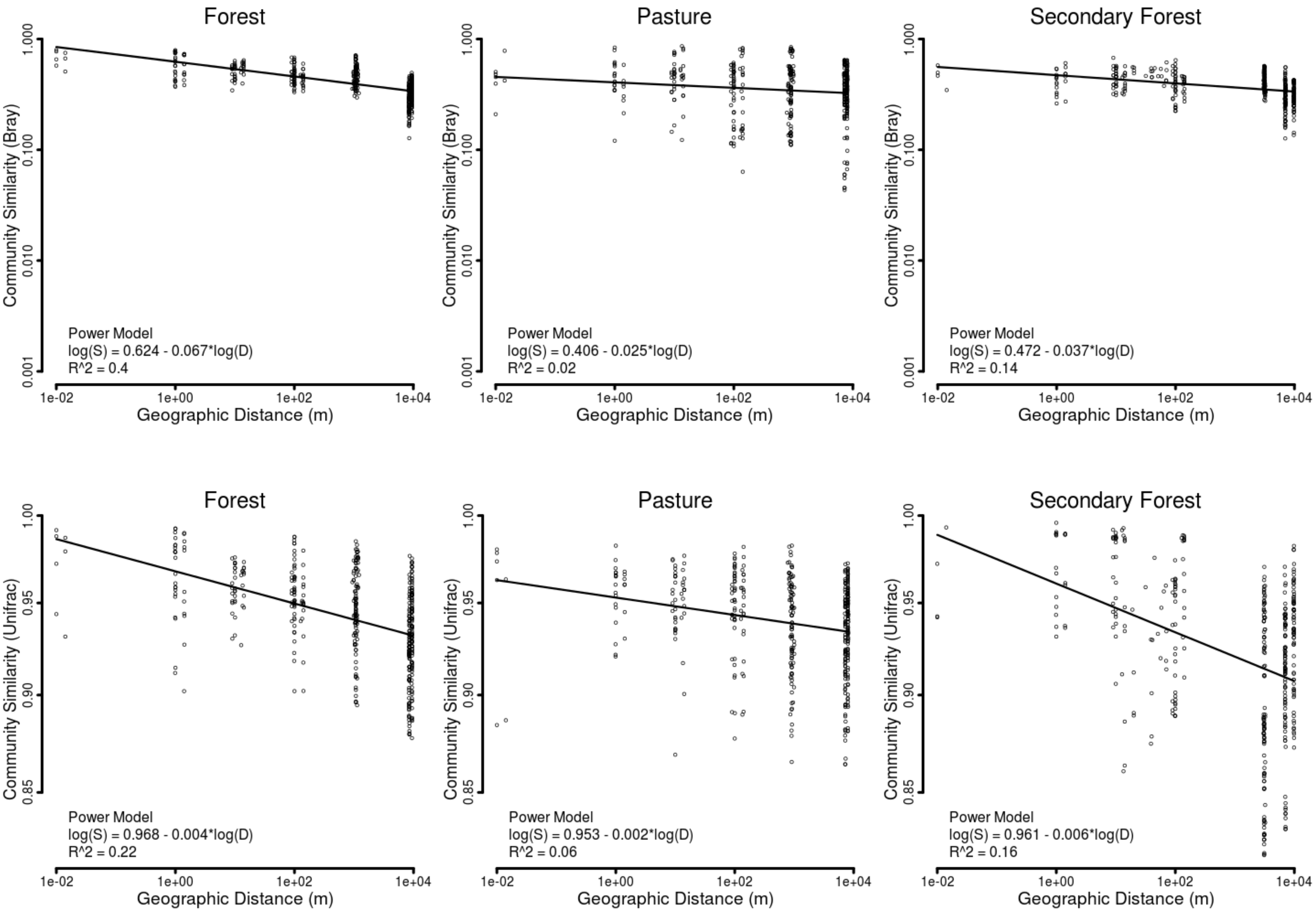


Fig. S2C

97% protein Bray-Curtis and unifrac metrics



Forest

Power Model
log(S) = 0.624 - 0.067*log(D)
R^2 = 0.4

Pasture

Power Model
log(S) = 0.406 - 0.025*log(D)
R^2 = 0.02

Secondary Forest

Power Model
log(S) = 0.472 - 0.037*log(D)
R^2 = 0.14

Forest

Power Model
log(S) = 0.968 - 0.004*log(D)
R^2 = 0.22

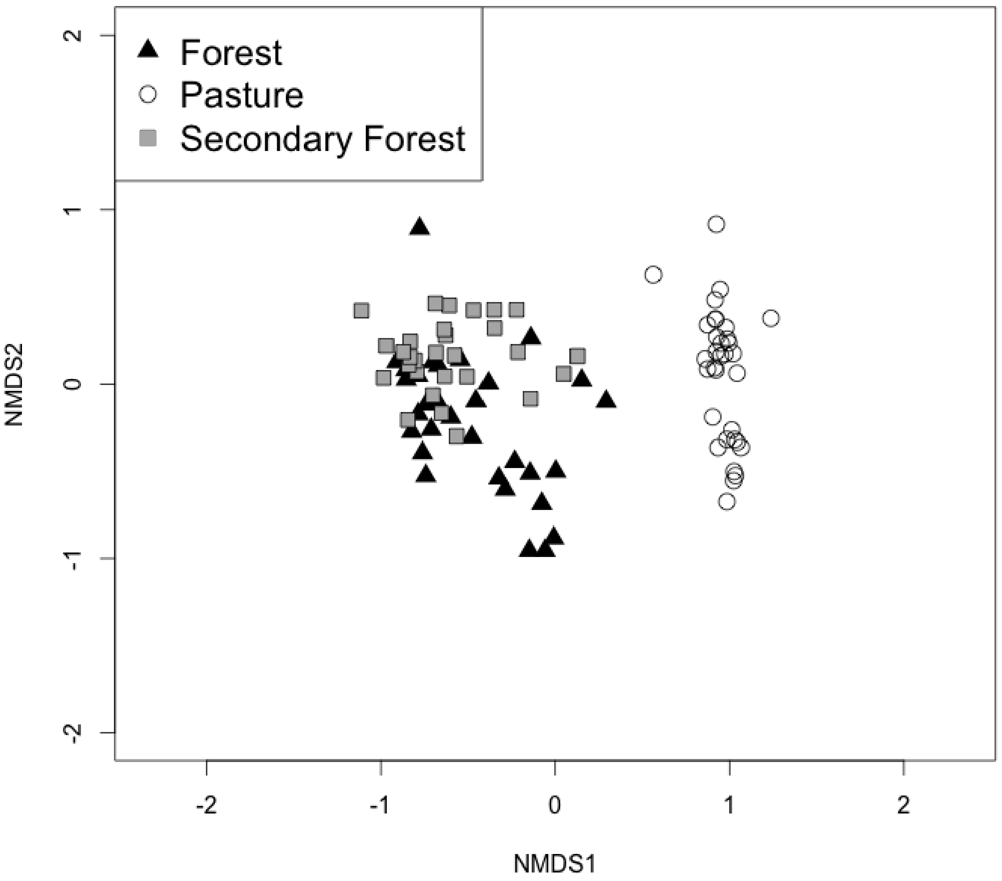
Pasture

Power Model
log(S) = 0.953 - 0.002*log(D)
R^2 = 0.06

Secondary Forest

Power Model
log(S) = 0.961 - 0.006*log(D)
R^2 = 0.16

Fig. S3 Analysis of similarity using subsampled data (135,000 *nifH* gene sequences) at 97% DNA similarity



ANOSIM statistic R: 0.7451

Significance: 0.001Dissimilarity: bray

Fig. S4 **Analysis of similarity using complete data (443,000 *nifH* gene sequences) at different DNA and protein similarity levels**

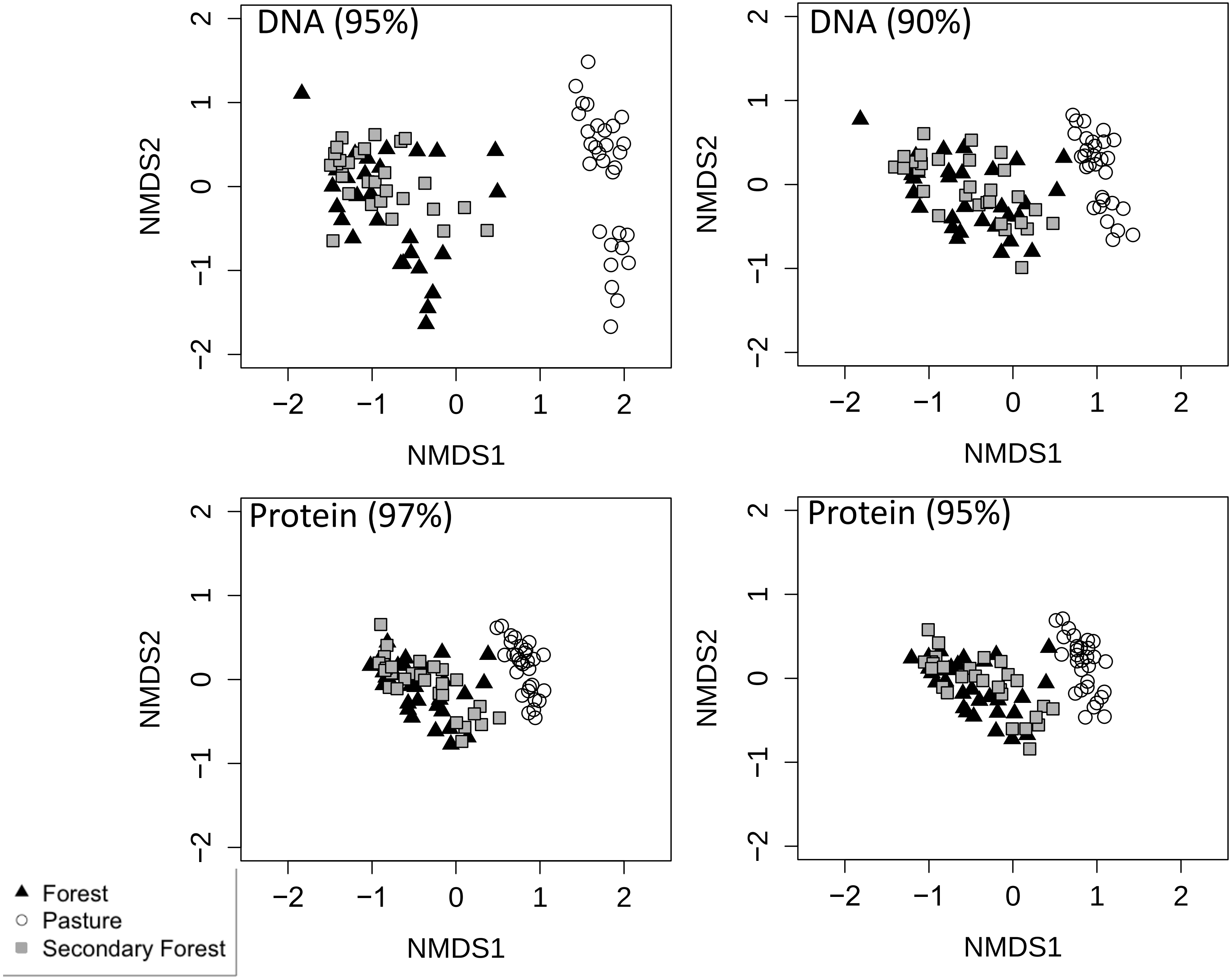


Fig S5: *NifH* gene abundance

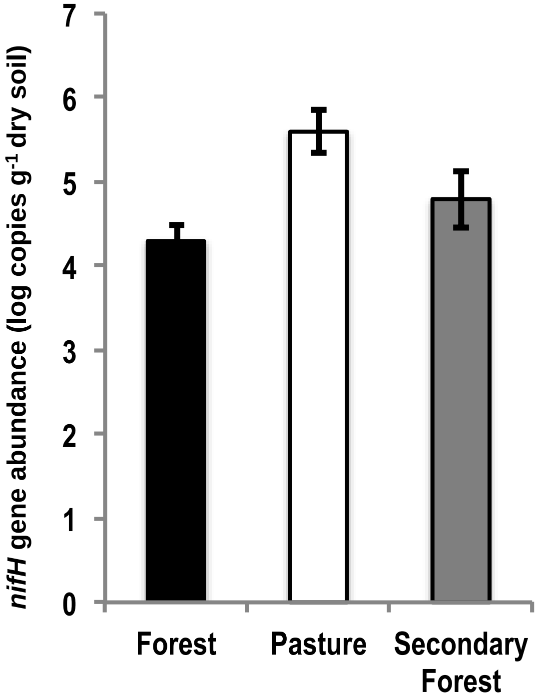
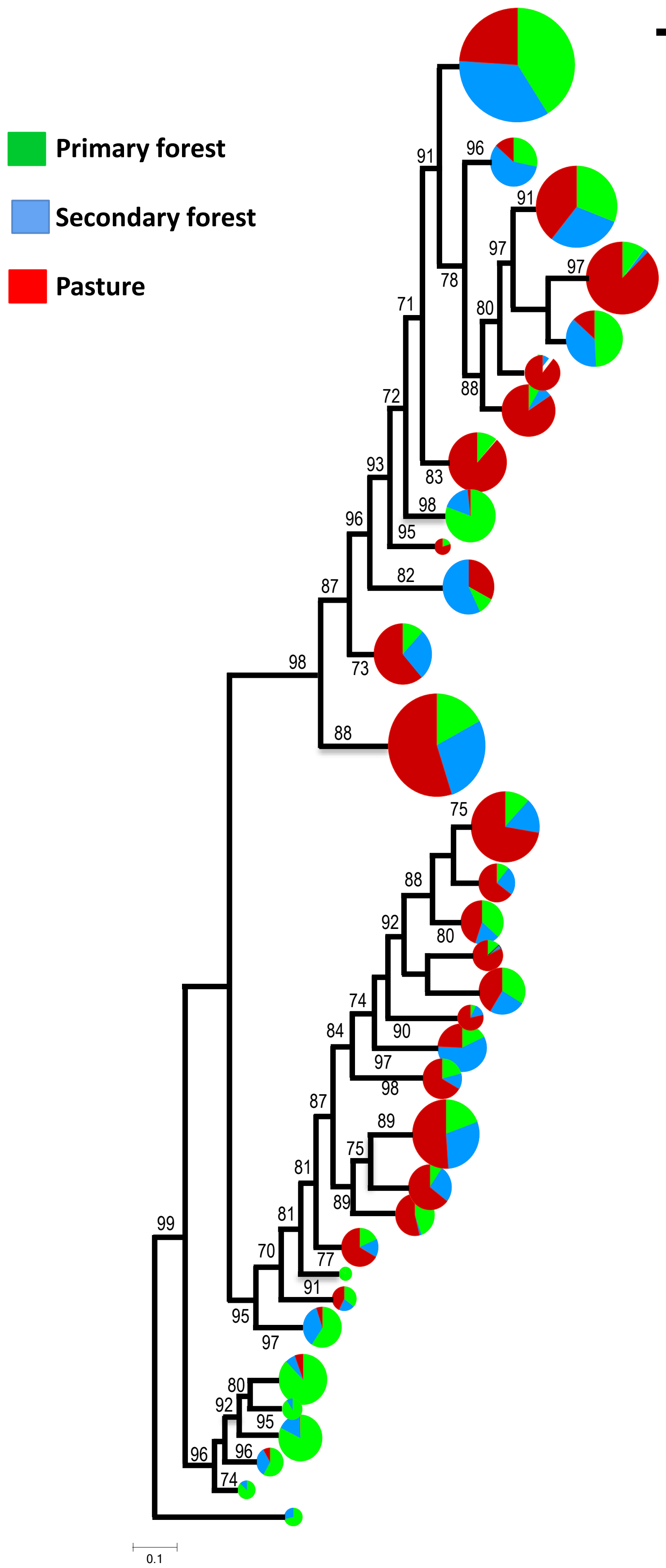


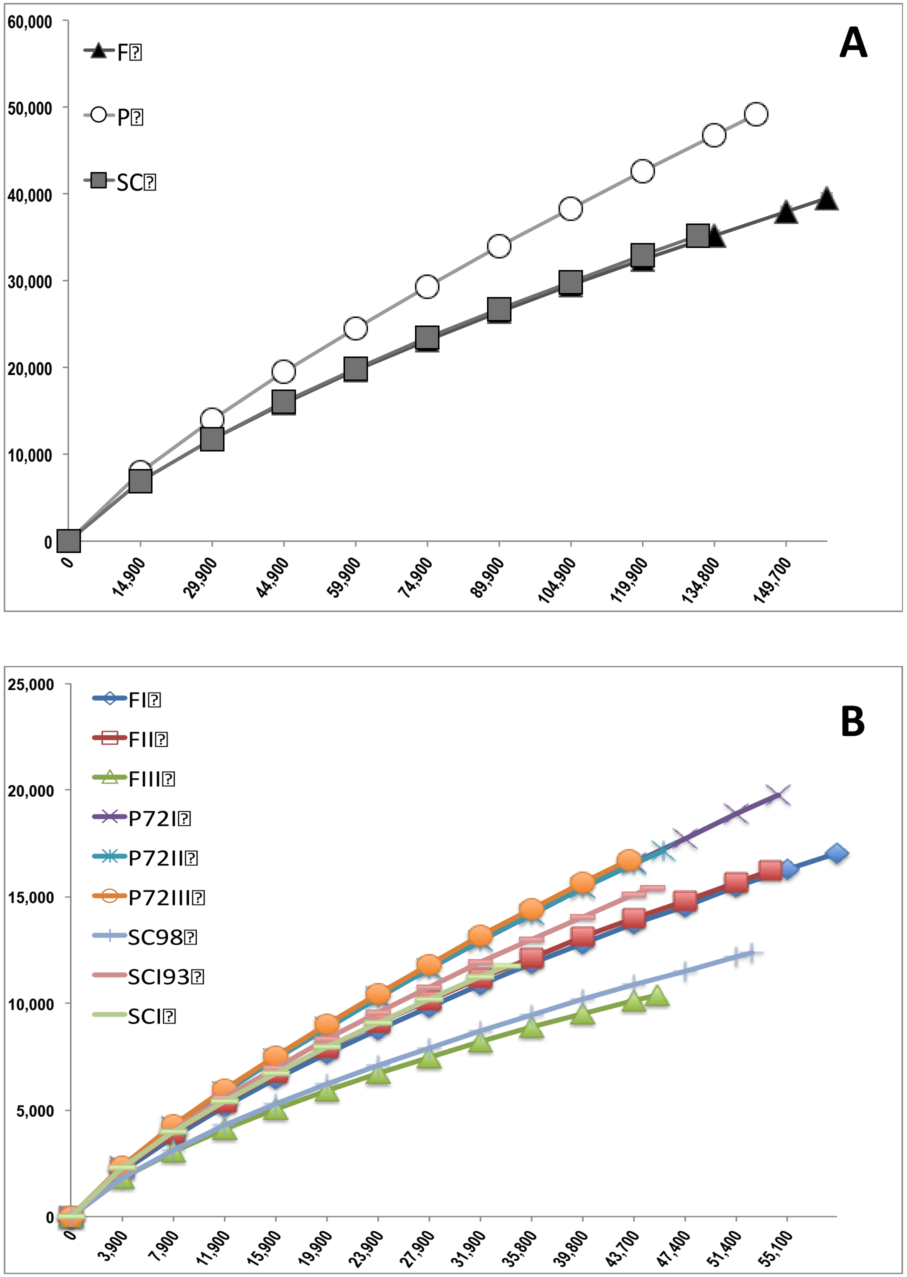
Fig S6: Variation in diazotrophic species



| Number of sequences | Taxonomic description | |
|---|---|---|
| 267,204 | *α-proteobacteria* | *nif*H cluster I |
| 551 | *α-proteobacteria* | |
| 17,800 | *β-proteobacteria* | |
| 4,272 | *γ-proteobacteria* | |
| 876 | *γ-proteobacteria* | |
| 112 | *Proteobacteria* (UC) | |
| 638 | *Proteobacteria* (UC) | |
| 1,097 | *Proteobacteria* (UC) | |
| 906 | *Paenibacillus* | |
| 15 | *Nostoc* | |
| 720 | *Frankia* | |
| 1,379 | *Firmicutes* | |
| 88,108 | *δ-proteobacteria* | |
| 3,800 | *Verrucomicrobium* | *nif*H cluster II |
| 159 | *Desulfobulbus (δ)* | |
| 288 | Uncultured | |
| 68 | *Desulfotomaculum* | |
| 432 | *Spirochetes* | |
| 41 | Uncultured | |
| 1,164 | Uncultured | |
| 168 | *Spirochetes* | |
| 2,958 | Uncultured | |
| 315 | *δ-proteobacteria* (UC) | |
| 185 | *Bacteroidetes* | |
| 159 | *Spirochetes (treponema)* | |
| 15 | *Clostridia* | |
| 41 | Uncultured | |
| 232 | *Clostridia* | |
| 647 | *γ-proteobacteria* | *nif*H cluster III |
| 32 | *α-proteobacteria* | |
| 445 | *Clostridia* | |
| 71 | *Clostridia* | |
| 23 | *Methanomicrobia* | |
| 21 | *Firmicutes (clostridium)* | *nif*H cluster IIV&V |

Fig. S7

97% DNA similarity



# Fig S8: A Spatially Explicit Sampling Scheme

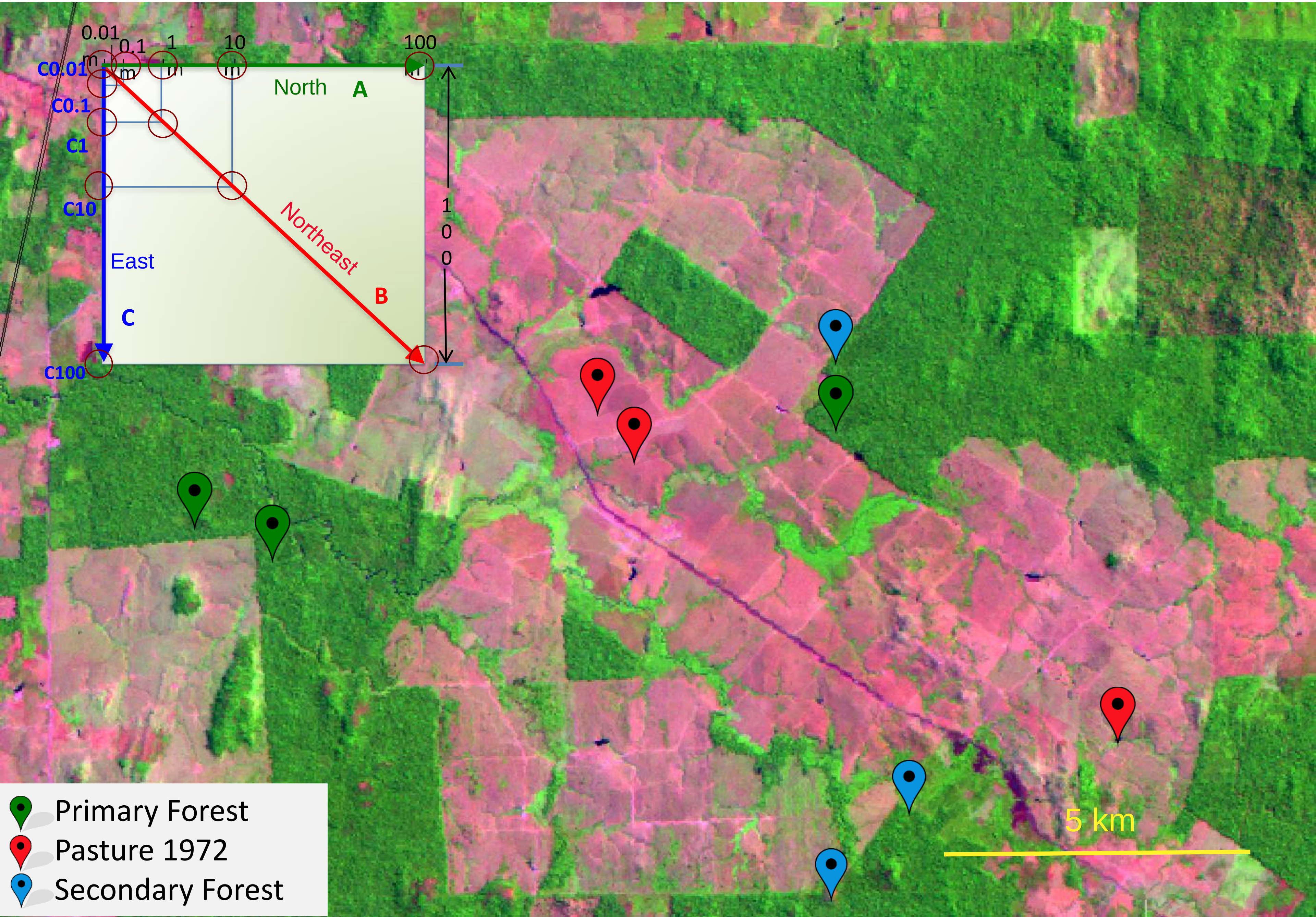


**Table S1.** Operational protein units (OPUs) and the closest matched sequences obtained through Wang et al., (2013) reference data. OPUs were clustered at 88% similarity.

| Cluster | OPU (No sequences) | Accession No. |
|---|---|---|
| **NifH_typeI** | *Alphaproteobacteria* (267,204) | *Zymomonas mobilis* (ACQU01000014), Magnetospirillum magneticum (AP007255), Methylocystis sp. (AEVM01000015), *Novosphingobium nitrogenifigens* (326209574), *Rhizobium* 1 encoding nitrogenase (152287), *Bradyrhizobium* sp. *ORS278* (146195046), *Beijerinckia indica* (182633352), *Azospirillum amazonense* Y2 (346917376), *Rhizobium* sp ANU289 (169750), *Hyphomicrobium* sp. MC1 (337761014), Unidentified bacterium nif cluster (13435000), *Bradyrhizobium japonicum* (EF394157), *Bradyrhizobium* sp. SEMIA (HQ259566), *Azospirillum* (DQ787332), *Methylocapsa aurea* (FN433472), *Bradyrhizobium yuanmingense* (EF394167), uncultured bacterium (EU912866) |
| | *Alphaproteobacteria* (551) | *Azorhizobium doebereinerae* (FJ223129), |
| | *Betaproteobacteria* (17,800) | *Sideroxydans lithotrophicus* ES1 (CP001965), *Candidatu Accumulibacter phosphatis* (CP001715), *Azoarcus* sp. BH72 (AF200742), *Dechloromonas aromatic* RCB (CP000089) |
| | *Gammaproteobacteria* (4,272) | *Klebsiella pneumoniae* 342 (CP000964), *Halorhodospira halophila* (AB189641) |
| | *Gammaproteobacteria* (876) | *Thiorhodococcus drewsii* (AFWT01000026) |
| | UC-*proteobacteria* (111) | Uncultured *Proteobacteria* |
| | UC-*proteobacteria* (636) | Uncultured *Proteobacteria* |
| | *Alphaproteobacteria* (1,099) | *Rhodomicrobium vannielii* (CP002292) |
| | *Paenibacillus* (902) | *Paenibacillus graminis* (AB485747), *Paenibacillus polymyxa* (HM146187), *Paenibacillus azotofixans* (AJ299453), *Paenibacillus fujiensis* (AB489070), *Paenibacillus sabinae* (HM583798) |
| | Cyanobacteria (15) | *Nostoc* sp. PCC 7120 (17130219), *Nostoc azollae* (YP_003720733), *Anabaena* sp. (NC_003272) |
| | *Frankia* (718) | *Frankia* sp. CcI3 (CP000249), *Frankia* sp. CeF (AC035776), *Frankia* sp. Cc1. 17 (ACJ24362) |
| | *Firmicutes* (1,374) | *Desulfitobacterium hafniense* DCB2 (CP001336), *Desulfosporosinus orientis* DSM765 (CP003108) |
| | *Deltaproteobacteria* (88,108) | *Geobacter metallireducens* GS15 (CP000148), *Geobacter bemidjiensis* (CP001124), *Geobacter* sp. M18 (ACPJ01000029), *Pelobacter propionicus* (CP000482), *Anaeromyxobacter* sp Fw109-5 (CP000769), *Anaeromyxobacter* sp. K (CP001131), *Desulfuromonas acetoxidans* DSM 684 (AAEW02000007), *Geobacter lovleyi*_SZ (CP001089),<br>*Geobacter daltonii* (YP_002538026), *Anaeromyxobacter* sp.(YP_001380211), uncultured bacterium (AY601069), uncultured bacterium (AY601072), *Geobacter uraniireducens* (NC_009483) |
| **NifH_typeII** | *Verrucomicrobium* (3,800) | *Opitutaceae bacterium* TAV2 (224804006), *Verrucomicrobiae bacterium*_DG1235 (198257712), *Coraliomargarita akajimensis* DSM45221 (CP001998), *Opitutaceae bacterium* TAV5(ZP_09596372) |
| | *Deltaproteobacteria* (*Desulfobulbus*) (158) | *Desulfobulbus propionicus* DSM 2032 (CP002364) |
| | Uncultured (287) | None |
| | *Firmicutes* (*Desulfotomaculum)* (67) | *Desulfobacca acetoxidans*_DSM_11109 (CP002629) |
| | *Spirochetes* (431) | *Spirochaeta thermophila* DSM6192 (CP001698), *Syntrophobacter fumaroxidans* MPOB (CP000478) |
| | Uncultured (41) | Uncultured methanogenic archaeon_RC-I (AM114193) |
| | Uncultured (1160) | None |
| | *Spirochetes* (167) | *Spirochaeta smaragdinae* DSM11293 (CP002116) |
| | Uncultured (2,962) | None |
| | *Deltaproteobacteria* (314) | *Deltaproteobacterium* NaphS2 (300447381) |
| | *Bacteroidetes* (185) | *Paludibacter propionicis* WB4 (CP002345) |
| | *Spirochetes* (treponema) (158) | *Treponema primitia* ZAS-2 (CP001843), *Treponema azotonutricium* ZAS-9 (CP001841) |
| | *Clostridia* (15) | *Clostridium thermocellum* (CP000568) |
| | Uncultured 41 | None |
| | *Clostridia* (231) | *Clostridium lentocellum* DSM 5427 (296249647), *Clostridium cellobioparum* (U59414),<br>*Clostridium papyrosolvens* DSM 2782 (ACXX01000015) |
| **NifH_typeIII** | *Gammaproteobacteria* (645) | *Thiorhodococcus drewsii* AZ1 (343918744), *Dickeya dadantii* Ech703 (CP001654) |
| | *Alphaproteobacteria* (32) | *Zymomonas mobilis* subsp pomaceae (CP002865) |
| | *Clostridia* (445) | *Clostridium pasteurianum* (40593), *Desulfosporosinus* sp OT (344326083), *Clostridium* sp. (357172598), *Chloroherpeton thalassium* ATCC 35110 (CP001100) |
| | *Clostridia* (70) | *Syntrophothermus lipocalidus* DSM 12680 (CP002048) |
| | Methanomicrobia (23) | *Methanosarcina acetivorans* str C2A (19915043), *Ethanolins harbinense* YUAN-3 (289520023) |
| **NifH_typeIV & V** | *Firmicutes (clostridia* (21) | *Clostridium cellulolyticum* H10 (NC_011898), *Geobacter sp* FRC-32 (CP001390), *Syntrophobotulus glycolicus* DSM8271 (CP002547), *Clostridiales genomosp* BVAB3 (CP001850), *Clostridium kluyveri* DSM 555 (CP000673), *Rhodopseudomonas palustris* DX1 (283578518) |

Table S2: Soil physiochemical characteristics of thee land-use systems in the Amazon.

| sample | pH | OM | **C** | N | C/N | P | S | K | Ca | Mg | Al | H+Al | SB | T | V | m | B | Cu | Fe | Mn | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FIA001 | 4.8 | 45 | 1.586 | 0.159 | 9.956 | 17 | 10 | 1.6 | 48 | 14 | 1 | 22 | 63.6 | 85.6 | 74 | 2 | 0.22 | 0.9 | 6 | 61.6 | 13 |
| FIA1 | 4.8 | 45 | 1.586 | 0.159 | 9.956 | 17 | 10 | 1.6 | 48 | 14 | 1 | 22 | 63.6 | 85.6 | 74 | 2 | 0.22 | 0.9 | 6 | 61.6 | 13 |
| FIA10 | 5.5 | 28 | 3.171 | 0.293 | 10.84 | 9 | 9 | 1.3 | 56 | 20 | 0.01 | 15 | 77.3 | 92.3 | 84 | 0 | 0.2 | 1 | 50 | 40.5 | 3.1 |
| FIA100 | 4.6 | 33 | 5.567 | 0.401 | 13.882 | 11 | 10 | 2.6 | 47 | 12 | 1 | 34 | 61.6 | 95.6 | 64 | 2 | 0.21 | 1.5 | 45 | 77.7 | 5.3 |
| FIB1 | 5.6 | 34 | 1.851 | 0.156 | 11.839 | 9 | 10 | 1.4 | 36 | 11 | 0.01 | 13 | 48.4 | 61.4 | 79 | 0 | 0.2 | 1 | 98 | 47.9 | 3.7 |
| FIB10 | 4.8 | 28 | 2.25 | 0.18 | 12.53 | 10 | 11 | 1.6 | 49 | 15 | 1 | 31 | 65.6 | 96.6 | 68 | 2 | 0.2 | 0.7 | 46 | 36.9 | 2.2 |
| FIB100 | 4.8 | 30 | 1.603 | 0.152 | 10.543 | 9 | 13 | 1 | 31 | 13 | 2 | 34 | 45 | 79 | 57 | 4 | 0.15 | 1.8 | 115 | 162.8 | 5.7 |
| FIC01 | 5.4 | 25 | 2.364 | 0.193 | 12.271 | 6 | 9 | 1 | 16 | 5 | 2 | 18 | 22 | 40 | 55 | 8 | 0.2 | 1.1 | 104 | 57.3 | 2.9 |
| FIC1 | 5.4 | 25 | 2.364 | 0.193 | 12.271 | 6 | 9 | 1 | 16 | 5 | 2 | 18 | 22 | 40 | 55 | 8 | 0.2 | 1.1 | 104 | 57.3 | 2.9 |
| FIC100 | 4.1 | 9 | 4.042 | 0.353 | 11.457 | 22 | 11 | 3 | 130 | 35 | 2 | 22 | 168 | 190 | 88 | 1 | 0.26 | 1.6 | 39 | 84.5 | 8 |
| FIIA001 | 3.9 | 16 | 1.105 | 0.076 | 14.497 | 5 | 9 | 0.8 | 10 | 3 | 1 | 31 | 13.8 | 44.8 | 31 | 7 | 0.19 | 0.9 | 49 | 42.7 | 0.8 |
| FIIA01 | 3.9 | 16 | 1.105 | 0.076 | 14.497 | 5 | 9 | 0.8 | 10 | 3 | 1 | 31 | 13.8 | 44.8 | 31 | 7 | 0.19 | 0.9 | 49 | 42.7 | 0.8 |
| FIIA1 | 3.9 | 16 | 1.105 | 0.076 | 14.497 | 5 | 9 | 0.8 | 10 | 3 | 1 | 31 | 13.8 | 44.8 | 31 | 7 | 0.19 | 0.9 | 49 | 42.7 | 0.8 |
| FIIA100 | 3.6 | 17 | 1.145 | 0.104 | 11.028 | 5 | 11 | 0.6 | 7 | 2 | 3 | 31 | 9.6 | 40.6 | 24 | 24 | 0.24 | 1.9 | 71 | 94 | 1.1 |
| FIIB1 | 4.5 | 15 | 0.781 | 0.068 | 11.536 | 5 | 9 | 0.4 | 16 | 4 | 6 | 18 | 20.4 | 38.4 | 53 | 23 | 0.2 | 0.8 | 32 | 34 | 0.5 |
| FIIB10 | 4.9 | 29 | 3.62 | 0.195 | 18.588 | 8 | 9 | 6 | 20 | 12 | 1 | 16 | 38 | 54 | 70 | 3 | 0.22 | 0.8 | 39 | 75.2 | 1 |
| FIIB100 | 5.2 | 36 | 1.275 | 0.095 | 13.422 | 9 | 12 | 1.8 | 43 | 10 | 0.01 | 18 | 54.8 | 72.8 | 75 | 0 | 0.32 | 1.3 | 45 | 73.4 | 1.3 |
| FIIC01 | 4.1 | 15 | 0.946 | 0.044 | 21.464 | 5 | 8 | 1.4 | 10 | 3 | 1 | 25 | 14.4 | 39.4 | 37 | 6 | 0.2 | 0.8 | 43 | 43.4 | 0.4 |
| FIIC1 | 4.1 | 15 | 0.946 | 0.044 | 21.464 | 5 | 8 | 1.4 | 10 | 3 | 1 | 25 | 14.4 | 39.4 | 37 | 6 | 0.2 | 0.8 | 43 | 43.4 | 0.4 |
| FIIC10 | 4.6 | 18 | 1.245 | 0.116 | 10.735 | 6 | 8 | 0.9 | 17 | 5 | 2 | 18 | 22.9 | 40.9 | 56 | 8 | 0.21 | 0.9 | 39 | 83.8 | 0.5 |
| FIIC100 | 4.2 | 19 | 1.905 | 0.095 | 20.064 | 6 | 9 | 0.6 | 20 | 3 | 1 | 25 | 23.6 | 48.6 | 49 | 4 | 0.23 | 1.2 | 57 | 29.5 | 0.6 |
| FIIIA001 | 3.9 | 13 | 0.658 | 0.044 | 14.997 | 4 | 8 | 1 | 4 | 2 | 2 | 20 | 7 | 27 | 26 | 22 | 0.18 | 0.6 | 36 | 26 | 0.5 |
| FIIIA01 | 3.9 | 13 | 0.658 | 0.044 | 14.997 | 4 | 8 | 1 | 4 | 2 | 2 | 20 | 7 | 27 | 26 | 22 | 0.18 | 0.6 | 36 | 26 | 0.5 |
| FIIIA1 | 3.9 | 13 | 0.658 | 0.044 | 14.997 | 4 | 8 | 1 | 4 | 2 | 2 | 20 | 7 | 27 | 26 | 22 | 0.18 | 0.6 | 36 | 26 | 0.5 |
| FIIIA100 | 4.4 | 14 | 0.306 | 0.015 | 20.569 | 7 | 9 | 2.8 | 7 | 3 | 1 | 11 | 12.8 | 23.8 | 54 | 7 | 0.18 | 0.3 | 26 | 12.2 | 0.7 |
| FIIIB1 | 3.8 | 12 | 0.7 | 0.043 | 16.475 | 5 | 9 | 0.6 | 5 | 1 | 1 | 20 | 6.6 | 26.6 | 25 | 13 | 0.2 | 0.9 | 45 | 27.8 | 0.5 |
| FIIIB10 | 4.2 | 16 | 0.513 | 0.022 | 23.825 | 4 | 8 | 0.4 | 8 | 1 | 6 | 13 | 9.4 | 22.4 | 42 | 39 | 0.16 | 0.7 | 62 | 23.7 | 1 |
| FIIIB100 | 4.2 | 8 | 0.486 | 0.02 | 24.41 | 4 | 8 | 0.4 | 1 | 1 | 1 | 15 | 2.4 | 17.4 | 14 | 29 | 0.18 | 0.1 | 64 | 2 | 0.4 |
| FIIIC01 | 3.7 | 10 | 0.642 | 0.041 | 15.827 | 4 | 9 | 0.6 | 2 | 1 | 2 | 11 | 3.6 | 14.6 | 25 | 36 | 0.22 | 0.8 | 43 | 24.1 | 0.3 |
| FIIIC1 | 3.7 | 10 | 0.642 | 0.041 | 15.827 | 4 | 9 | 0.6 | 2 | 1 | 2 | 11 | 3.6 | 14.6 | 25 | 36 | 0.22 | 0.8 | 43 | 24.1 | 0.3 |
| FIIIC10 | 3.7 | 19 | 0.565 | 0.024 | 23.852 | 6 | 8 | 1.2 | 17 | 4 | 7 | 22 | 22.2 | 44.2 | 50 | 24 | 0.18 | 0.6 | 7 | 15.2 | 1 |
| SC93A01 | 4.5 | 28 | 1.09 | 0.073 | 14.917 | 4 | 9 | 1.5 | 14 | 8 | 2 | 31 | 23.5 | 54.5 | 43 | 8 | 0.16 | 1.6 | 87 | 50.9 | 1.7 |
| SC93A10 | 4 | 19 | 2.183 | 0.133 | 16.434 | 4 | 8 | 1.4 | 6 | 3 | 4 | 31 | 10.4 | 41.4 | 25 | 28 | 0.2 | 1 | 83 | 11.2 | 0.8 |
| SC93A20 | 3.9 | 35 | 1.86 | 0.14 | 13.285 | 8 | 8 | 1.8 | 10 | 7 | 6 | 47 | 18.8 | 65.8 | 29 | 24 | 0.18 | 0.8 | 133 | 16 | 1.3 |
| SC93B1 | 4.2 | 28 | 2.835 | 0.177 | 15.999 | 6 | 8 | 1 | 19 | 7 | 1 | 31 | 27 | 58 | 47 | 4 | 0.15 | 0.8 | 88 | 41.5 | 2.4 |
| SC93B40 | 3.6 | 51 | 1.758 | 0.134 | 13.102 | 9 | 10 | 1.1 | 6 | 4 | 13 | 80 | 11.1 | 91.1 | 12 | 54 | 0.16 | 0.8 | 191 | 28.2 | 4.8 |
| SC93C01 | 4.1 | 32 | 2.438 | 0.158 | 15.436 | 6 | 9 | 1.3 | 16 | 7 | 3 | 42 | 24.3 | 66.3 | 37 | 11 | 0.12 | 0.8 | 96 | 64 | 2.8 |
| SC93C1 | 4.1 | 32 | 2.438 | 0.158 | 15.436 | 6 | 9 | 1.3 | 16 | 7 | 3 | 42 | 24.3 | 66.3 | 37 | 11 | 0.12 | 0.8 | 96 | 64 | 2.8 |
| SC93C10 | 4.3 | 17 | 1.179 | 0.093 | 12.723 | 4 | 9 | 4.1 | 13 | 7 | 1 | 22 | 24.1 | 46.1 | 52 | 4 | 0.15 | 1.1 | 62 | 25.2 | 3.4 |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SC93C50 | 4.3 | 25 | 2.471 | 0.139 | 17.78 | 5 | 8 | 1.5 | 14 | 7 | 2 | 31 | 22.5 | 53.5 | 42 | 8 | 0.18 | 0.9 | 109 | 14.8 | 26.3 |
| SC98A001 | 4 | 19 | 0.795 | 0.069 | 11.5 | 6 | 13 | 0.9 | 7 | 3 | 4 | 34 | 10.9 | 44.9 | 24 | 27 | 0.22 | 0.7 | 41 | 259.8 | 0.8 |
| SC98A01 | 4 | 19 | 0.795 | 0.069 | 11.5 | 6 | 13 | 0.9 | 7 | 3 | 4 | 34 | 10.9 | 44.9 | 24 | 27 | 0.22 | 0.7 | 41 | 259.8 | 0.8 |
| SC98A1 | 4 | 19 | 0.795 | 0.069 | 11.5 | 6 | 13 | 0.9 | 7 | 3 | 4 | 34 | 10.9 | 44.9 | 24 | 27 | 0.22 | 0.7 | 41 | 259.8 | 0.8 |
| SC98A10 | 3.7 | 14 | 1.005 | 0.081 | 12.474 | 5 | 14 | 0.8 | 3 | 2 | 7 | 34 | 5.8 | 39.8 | 15 | 55 | 0.24 | 0.8 | 42 | 205 | 0.5 |
| SC98A100 | 4.4 | 21 | 0.797 | 0.056 | 14.353 | 5 | 9 | 0.8 | 13 | 4 | 1 | 28 | 17.8 | 45.8 | 39 | 5 | 0.16 | 0.4 | 44 | 134.4 | 0.5 |
| SC98B1 | 3.9 | 14 | 0.766 | 0.064 | 12.025 | 5 | 17 | 0.7 | 4 | 2 | 4 | 31 | 6.7 | 37.7 | 18 | 37 | 0.2 | 0.6 | 35 | 223.2 | 0.4 |
| SC98B10 | 3.9 | 15 | 0.847 | 0.065 | 13.087 | 5 | 19 | 0.7 | 1 | 1 | 5 | 31 | 2.7 | 33.7 | 8 | 65 | 0.21 | 0.5 | 35 | 207.4 | 0.4 |
| SC98B100 | 4.4 | 15 | 1.261 | 0.083 | 15.137 | 3 | 9 | 0.8 | 9 | 2 | 1 | 31 | 11.8 | 42.8 | 28 | 8 | 0.16 | 0.5 | 41 | 55 | 0.3 |
| SC98C01 | 3.9 | 11 | 0.919 | 0.077 | 11.872 | 4 | 21 | 0.6 | 3 | 1 | 6 | 31 | 4.6 | 35.6 | 13 | 57 | 0.21 | 0.6 | 34 | 182.4 | 10.9 |
| SC98C1 | 3.9 | 11 | 0.919 | 0.077 | 11.872 | 4 | 21 | 0.6 | 3 | 1 | 6 | 31 | 4.6 | 35.6 | 13 | 57 | 0.21 | 0.6 | 34 | 182.4 | 10.9 |
| SC98C10 | 4.3 | 15 | 0.843 | 0.069 | 12.207 | 6 | 25 | 2.3 | 8 | 3 | 3 | 25 | 13.3 | 38.3 | 35 | 18 | 0.23 | 0.9 | 33 | 189.2 | 4.7 |
| SC98C100 | 3.7 | 11 | 0.725 | 0.052 | 14.062 | 5 | 13 | 0.8 | 2 | 1 | 7 | 25 | 3.8 | 28.8 | 13 | 65 | 0.21 | 0.7 | 36 | 151.4 | 0.4 |
| SCIA1 | 4.7 | 33 | 1.21 | 0.11 | 11.03 | 6 | 12 | 1.3 | 15 | 9 | 1 | 31 | 25.3 | 56.3 | 45 | 4 | 0.17 | 1.3 | 69 | 59.8 | 6.3 |
| SCIA10 | 4.4 | 33 | 1.68 | 0.15 | 11.1 | 6 | 12 | 1.6 | 20 | 8 | 1 | 34 | 29.6 | 63.6 | 47 | 3 | 0.19 | 1.6 | 95 | 82.8 | 1 |
| SCIA100 | 3.8 | 17 | 1.38 | 0.12 | 11.79 | 5 | 11 | 0.5 | 3 | 2 | 5 | 31 | 5.5 | 36.5 | 15 | 48 | 0.2 | 1.3 | 62 | 7.8 | 0.3 |
| SCIB1 | 4.6 | 37 | 3.6 | 0.22 | 16.61 | 6 | 11 | 1.8 | 25 | 15 | 1 | 25 | 41.8 | 66.8 | 63 | 2 | 0.18 | 1.4 | 69 | 34.9 | 1 |
| SCIB10 | 4.4 | 26 | 2.16 | 0.16 | 13.39 | 6 | 9 | 1.1 | 12 | 8 | 1 | 25 | 21.1 | 46.1 | 46 | 5 | 0.18 | 1.4 | 59 | 41.3 | 1.5 |
| SCIB100 | 4.1 | 17 | 1.62 | 0.12 | 13.24 | 4 | 12 | 0.8 | 7 | 3 | 3 | 42 | 10.8 | 52.8 | 20 | 22 | 0.2 | 1.2 | 64 | 13.3 | 0.3 |
| SCIC1 | 4.6 | 43 | 3.33 | 0.21 | 15.98 | 6 | 13 | 1.5 | 20 | 13 | 1 | 28 | 34.5 | 62.5 | 55 | 3 | 0.15 | 1.3 | 48 | 34.2 | 4.3 |
| SCIC100 | 4.3 | 19 | 1.13 | 0.1 | 11.8 | 5 | 9 | 1.7 | 8 | 5 | 1 | 28 | 14.7 | 42.7 | 34 | 6 | 0.2 | 1 | 64 | 65 | 0.5 |
| P72IA001 | 4 | 25 | 1.2 | 0.07 | 17.91 | 5 | 8 | 1.3 | 9 | 2 | 2 | 15 | 12.3 | 27.3 | 45 | 14 | 0.17 | 1 | 104 | 26.2 | 1 |
| P72IA01 | 4 | 25 | 1.2 | 0.07 | 17.91 | 5 | 8 | 1.3 | 9 | 2 | 2 | 15 | 12.3 | 27.3 | 45 | 14 | 0.17 | 1 | 104 | 26.2 | 1 |
| P72IA1 | 4 | 25 | 1.2 | 0.07 | 17.91 | 5 | 8 | 1.3 | 9 | 2 | 2 | 15 | 12.3 | 27.3 | 45 | 14 | 0.17 | 1 | 104 | 26.2 | 1 |
| P72IA10 | 4.6 | 28 | 1.68 | 0.11 | 15.67 | 4 | 8 | 1.5 | 9 | 2 | 2 | 20 | 12.5 | 32.5 | 38 | 14 | 0.17 | 1.4 | 71 | 26.8 | 1.6 |
| P72IA100 | 4.6 | 28 | 2.21 | 0.14 | 16.17 | 3 | 8 | 0.5 | 16 | 2 | 2 | 22 | 18.5 | 40.5 | 46 | 10 | 0.14 | 1.8 | 95 | 44 | 1.8 |
| P72IB1 | 4.6 | 29 | 1.63 | 0.11 | 14.98 | 3 | 8 | 1.4 | 11 | 3 | 1 | 22 | 15.4 | 37.4 | 41 | 6 | 0.14 | 1.3 | 105 | 26.4 | 1.5 |
| P72IB10 | 4.6 | 29 | 2.16 | 0.113 | 19.113 | 3 | 8 | 1.7 | 10 | 3 | 1 | 22 | 14.7 | 36.7 | 40 | 6 | 0.19 | 1.5 | 65 | 30.9 | 2 |
| P72IB100 | 4.6 | 23 | 1.25 | 0.072 | 17.45 | 3 | 8 | 1 | 9 | 3 | 1 | 22 | 13 | 35 | 37 | 7 | 0.21 | 1 | 67 | 33 | 1.1 |
| P72IC01 | 4.8 | 30 | 1.645 | 0.107 | 15.398 | 4 | 6 | 1.9 | 10 | 4 | 1 | 22 | 15.9 | 37.9 | 42 | 6 | 0.18 | 1 | 112 | 26 | 1.5 |
| P72IC1 | 4.8 | 30 | 1.645 | 0.107 | 15.398 | 4 | 6 | 1.9 | 10 | 4 | 1 | 22 | 15.9 | 37.9 | 42 | 6 | 0.18 | 1 | 112 | 26 | 1.5 |
| P72IC10 | 4.7 | 29 | 1.63 | 0.096 | 16.91 | 6 | 8 | 1.3 | 13 | 4 | 1 | 25 | 18.3 | 43.3 | 42 | 5 | 0.18 | 1.3 | 87 | 33.3 | 2.2 |
| P72IC100 | 4.5 | 29 | 1.755 | 0.11 | 16.011 | 4 | 8 | 1 | 10 | 3 | 1 | 25 | 14 | 39 | 36 | 7 | 0.15 | 0.8 | 98 | 26.1 | 1.6 |
| P72IIA001 | 4.5 | 25 | 2.071 | 0.135 | 15.382 | 3 | 8 | 0.7 | 7 | 2 | 2 | 28 | 9.7 | 37.7 | 26 | 17 | 0.14 | 0.7 | 47 | 23.9 | 0.9 |
| P72IIA01 | 4.5 | 25 | 2.071 | 0.135 | 15.382 | 3 | 8 | 0.7 | 7 | 2 | 2 | 28 | 9.7 | 37.7 | 26 | 17 | 0.14 | 0.7 | 47 | 23.9 | 0.9 |
| P72IIA1 | 4.5 | 25 | 2.071 | 0.135 | 15.382 | 3 | 8 | 0.7 | 7 | 2 | 2 | 28 | 9.7 | 37.7 | 26 | 17 | 0.14 | 0.7 | 47 | 23.9 | 0.9 |
| P72IIB10 | 4.3 | 26 | 1.885 | 0.141 | 13.395 | 3 | 8 | 0.8 | 6 | 3 | 2 | 25 | 9.8 | 34.8 | 28 | 17 | 0.17 | 0.8 | 65 | 24.3 | 1.2 |
| P72IIB100 | 5 | 33 | 2.038 | 0.128 | 15.928 | 4 | 12 | 7 | 13 | 8 | 0.01 | 18 | 28 | 46 | 61 | 0 | 0.18 | 1 | 96 | 48 | 2.1 |
| P72IIC01 | 4.3 | 30 | 1.98 | 0.133 | 14.927 | 3 | 8 | 1.2 | 8 | 3 | 3 | 28 | 12.2 | 40.2 | 30 | 20 | 0.18 | 0.8 | 57 | 24.9 | 1.3 |
| P72IIC10 | 4.8 | 44 | 3.532 | 0.203 | 17.387 | 6 | 8 | 1.2 | 23 | 5 | 1 | 31 | 29.2 | 60.2 | 49 | 3 | 0.22 | 1.5 | 88 | 51.2 | 4 |
| P72IIC100 | 4.4 | 33 | 2.544 | 0.172 | 14.834 | 4 | 7 | 1.7 | 9 | 4 | 3 | 31 | 14.7 | 45.7 | 32 | 17 | 0.2 | 1.3 | 77 | 55.8 | 1.5 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P72IIIA01 | 5.1 | 44 | 5.342 | 0.409 | 13.061 | 18 | 9 | 3.2 | 28 | 7 | 0.01 | 31 | 38.2 | 69.2 | 55 | 0 | 0.2 | 5.8 | 198 | 222 | 14 |
| P72IIIA1 | 5.1 | 44 | 5.342 | 0.409 | 13.061 | 18 | 9 | 3.2 | 28 | 7 | 0.01 | 31 | 38.2 | 69.2 | 55 | 0 | 0.2 | 5.8 | 198 | 222 | 14 |
| P72IIIA10 | 5.3 | 54 | 5.245 | 0.424 | 12.365 | 16 | 9 | 4.3 | 39 | 12 | 0.01 | 28 | 55.3 | 83.3 | 66 | 0 | 0.22 | 5.8 | 142 | 203 | 16.4 |
| P72IIIA100 | 5.1 | 49 | 5.186 | 0.452 | 11.474 | 10 | 9 | 3.5 | 27 | 7 | 0.01 | 31 | 37.5 | 68.5 | 55 | 0 | 0.22 | 5.6 | 70 | 193.4 | 9.4 |
| P72IIIB1 | 5.2 | 41 | 2.495 | 0.219 | 11.4 | 8 | 9 | 1.7 | 25 | 4 | 0.01 | 28 | 30.7 | 58.7 | 52 | 0 | 0.2 | 5.1 | 103 | 184.6 | 9.6 |
| P72IIIB100 | 5.3 | 50 | 7.476 | 0.581 | 12.873 | 29 | 15 | 4.6 | 33 | 14 | 0.01 | 28 | 51.6 | 79.6 | 65 | 0 | 0.22 | 5.4 | 128 | 171.6 | 12.3 |
| P72IIIC01 | 5.2 | 62 | 6.446 | 0.47 | 13.724 | 15 | 9 | 2.4 | 30 | 9 | 0.01 | 34 | 41.4 | 75.4 | 55 | 0 | 0.2 | 5.7 | 131 | 163.4 | 15.5 |
| P72IIIC1 | 5.2 | 62 | 6.446 | 0.47 | 13.724 | 15 | 9 | 2.4 | 30 | 9 | 0.01 | 34 | 41.4 | 75.4 | 55 | 0 | 0.2 | 5.7 | 131 | 163.4 | 15.5 |
| P72IIIC10 | 5.5 | 43 | 3.604 | 0.268 | 13.43 | 19 | 9 | 1.4 | 35 | 9 | 0.01 | 20 | 45.4 | 65.4 | 69 | 0 | 0.18 | 4.2 | 59 | 165.8 | 12.2 |
| P72IIIC100 | 5.3 | 50 | 3.043 | 0.276 | 11.045 | 11 | 13 | 1.7 | 23 | 8 | 0.01 | 31 | 32.7 | 63.7 | 51 | 0 | 0.21 | 5 | 255 | 174.8 | 7.9 |

**Measured soil variables:** OM, organic matter, C, carbon; N, nitrogen; pH, potential activity of hydrogen; P, phosphorus; S, sulfur; K, potassium; Ca, calcium; Mg, magnesium; Al, aluminum; $H^+$, hydrogen; CEC, potential cation exchange capacity; V, base saturation; $Al^+$ sat, aluminum saturation. C and N are expressed in $g.Kg^{-1}$ soil; P and S are expressed as mg $dm^{-3}$; K, Ca, Mg, Al and CEC are expressed in mmol $c.dm^{-3}$.

List of the 19 soil samples produced <1500 nifH gene sequences were not included in this study. Overall 89 soil samples were analyzed across three different land-use systems.

FIA01, FIC10, FIIA10, FIIIA10, FIIIC100
P72IB1, P72IIA10, P72IIA100, P72IIB1, P72C1, P72IIIA001, P72IIIB10
SC98B1, SCI93A001, SCI93B10, SCIC10,
SCI93A40, SCIA001, SCIA01