## Supplementary text

## Sequencing statistics

In total, 460,981 high-quality sequences were assigned to ITS2 and 1,054,333 to rbcL. There was considerable variation in the number of reads per sample, with the total number of high quality rbcL sequences varying from 3 to 56,981 and the number of ITS2 sequences ranging from 260 to 10,180 (excluding a single sample that contained zero sequences following quality control).

Excluding control samples, ITS2 reads included 179,450 grass sequences (Poaceae), assigned to 13 genera. Whilst not the focus of our study, the remaining 162,540 sequences consisted of 33 families and 31 genera of terrestrial plants. Of these families, 17 contained only a single genus and four contained reads which could not be identified confidently to genus level. Within the rbcL marker, 179,330 grass reads were assigned to 13 grass genera and the remaining 867,823 reads belonged to 68 families and 84 genera of terrestrial plants. Of these families, 33 contained only a single genus and 13 contained reads which could not be identified confidently to genus level.

## ITS2 and rbcL detect different grass species

The contrasting characteristics of the ITS2 and rbcL markers makes them an ideal pairing. The ITS2 marker shows high specificity between species but cannot detect all plants [38], whereas rbcL primers are highly universal but the marker shows lower resolution between closely related plants [39].

Of the grass genera identified, only four were present in both ITS2 and rbcL datasets: Dactylis, Lolium/Festuca, Anthoxanthum, and Avena. While the proportion of reads assigned to Lolium/Festuca and Anthoxanthum were correlated between the two markers (Lolium/Festuca (Lolium/Festuca: $\mathrm{t}_{72}=8.6$, adjusted p -value $<0.001, \mathrm{r}^{2}=0.5$, S4A Fig; Anthoxanthum: $\mathrm{t}_{72}=2.9$, adjusted p -value $=0.006, \mathrm{r}^{2}=0.09$, S 4 B Fig), this was not the case for Dactylis or Avena (S4C Fig; S4D Fig). However, both of the latter species were detected at
relatively low levels in both datasets, potentially increasing the degree of stochasticity introduced by library preparation [40].

## Positive and negative controls

Negative controls, with all reagents and no DNA were used to identify any crosscontamination. Of the six negative controls, four contained no reads following quality control filtering, one contained a single read and one contained nine reads in the rbcL database. None of the negative controls in the ITS2 dataset contained any reads.

Two positive control samples were also included, a grass positive control and an exotic plant positive control. Both sets of positive controls were diluted to $0.3 \mathrm{ng}^{\mathrm{l}^{-1}}$, similar to that of the aerial eDNA samples.

The grass positive control contained a mixture of fifty-two species of grass from herbarium collections held at the National Botanic Garden of Wales. The mixture of grasses contained thirty-three genera, with twenty-four of these genera represented by a single species and the remaining nine genera represented by between two and five species (S1 Table). Of the thirty-three genera in the grass positive control sample, four were detected across both markers, eight were detected by the rbcL marker and twelve by the ITS2 marker. The remaining sequences were too similar to be identified to genus level ( $27 \%$ and $37 \%$ of reads in the grass positive control samples could not be reliably assigned to genus level, using ITS2 and $r b c L$ markers respectively). However, three of the genera not detected the positive control samples, despite being included, were detected in airborne samples (Agrostis, Anthoxanthum, Alopecurus), likely reflecting higher local abundances of airborne pollen (S2 Table). The number of species in the grass positive control is much higher than the number of species predicted to contribute to airborne pollen concentrations according to phenological studies [41, 42]. Differences in taxon diversity between the grass positive control and the airborne samples will likely lead to differences in taxonomic assignment due to taxon-specific PCR amplification biases [43-45]. While sample coverage (i.e. number of reads) obtained for
the grass positive control samples was comparable to the airborne samples, the high diversity of the positive control and variation in the number of species between genera may have led to a higher likelihood of amplification for certain genera.

In order to check for cross-contamination between samples, an exotic plant positive control sample was used containing DNA extracted from twenty-one tropical tree species samples held at the National Botanic Garden of Wales. None of the genera identified in this positive control were present in the experimental samples.


70 S1 Fig. Map showing position of the six sampling sites. Contains OS data Crown copyright and database right (2018). Image Crown Copyright, 2018, The Met Office.


S2 Fig. Non-metric multidimensional scaling (NMDS) ordination of grass community similarity shows a strong effect of time on the overall community composition. Coloured circles indicate sampling sites. Site labels are abbreviated as follows: BNG = Bangor; EXE = Exeter; ING = Invergowrie; IOW = Isle of Wight; WOR = Worcester; YORK = York. Coloured circles indicate samples sites. Site labels abbreviated as follows: BNG = Bangor; EXE = Exeter; ING = Invergowrie; IOW = Isle of Wight; WOR = Worcester; YORK = York.

82 S3 Fig. Relative abundance of the five most abundant grasses at genus level, normalized according to airborne pollen concentration data. Relative abundances were calculated as a proportion of reads assigned to Poaceae, rather than of reads as a whole, then multiplied by mean pollen concentration across the three pooled days. Markers used to identify grass pollen are stated in the top panel label.

6 Due to errors in sampling equipment, only 4 weeks of samples were collected at the York sampling site. Sampling sites are indicated in the right panel label abbreviated as follows: BNG = Bangor; EXE = Exeter; ING = Invergowrie; IOW = Isle of Wight; WOR = Worcester; YORK = York.








Taxa






S4 Fig. Correlations between proportions of reads made up by the same genus in the two marker gene datasets. All four genera present in both datasets are shown: (A) Lolium/Festuca, (B)

2 Anthoxanthum, (C) Avena, and (D) Dactylis. For cases where there was a significant relationship between relative abundances in both datasets, black lines show the intercept and slope.

95

96


97 S5 Fig. Photograph of 1.5 ml microcentrifuge tubes mounted onto carousel on Burkard Automatic Multi-Vial Cyclone Sampler. Author provided.
A)

B)


S6 Fig. There is a strong relationship between the mean proportion of sequences and the variance of the proportion of sequences from each sampling site using both A) rbcL and B) ITS2 markers. Coloured circles denote sampling site. The plots were produced using the meanvar.plot function in the mvabund package in $R(21)$.
A)

B)


S7 Fig. Scatter plot of linear predictor values and the residuals output from the models selected to analyse the abundance data produced by the A) rbcL marker and B) ITS2 marker. Little pattern suggests that the models selected are plausible and the mean-variance assumption of the negative binomial regression is correct. Coloured circles denote different genera in the abundance data. The plots were produced using the plot.manyglm function in the mvabund package in $R$ (21).

122 S1 List Borneo plant taxa pooled for the exotic plant positive control.

Aglaia sp.
125 Antidesma sp.
126 Baccaurea stipulata
127 Cynometra sp.
128 Dalbergia sp.
129 Dehaasia sp.
130 Dillenia excelsa
131 Diospryos sp.
132 Kleinhovia hospita
133 Lagerstroemia sp.
134 Lophopyxis sp.
135 Madhuca dubardii
136 Mallotus muticus
137 Microcos crassifolia
138 Pternandra sp.
139 Pterospermum macrocarpum
140 Syzygium sp.
141 Uncaria sp.
142 Urophyllum sp.

Vatica sp.
Xylosma sp.

S1 Table. Grass species pooled for the Grass Positive Control at equal volumes.

| Grass positive control | Concentration of DNA <br> $(\mathrm{ng} / \mu \mathrm{l})$ |
| :--- | :--- |


| Agrostis canina | 0.121 |
| :---: | :---: |
| Agrostis capillaris | 1.29 |
| Agrostis gigantea | 9.48 |
| Agrostis stolonifera | 3.7 |
| Agrostis vinealis | 1.03 |
| Aira praecox | 0.8 |
| Alopecurus geniculatus | 1.18 |
| Alopecurus pratensis | 1.42 |
| Anisantha sterilis | 0.848 |
| Anthoxanthum odoratum | 0.804 |
| Arrhenatherum elatius | 1.36 |
| Brachypodium sylvaticum | 0.804 |
| Briza media | 2 |
| Bromopsis ramosa | 0.35 |
| Bromus hordeaceus | 0.098 |
| Catapodium rigidum | 3.96 |
| Cynosurus cristatus | 0.0736 |
| Dactylis glomerata | 13.8 |
| Danthonia decumbens | 1.34 |
| Deschampsia cespitosa | 2.52 |
| Deschampsia flexuosa | 0.648 |
| Elymus caninus | 1.62 |
| Elytrigia repens | 3.47 |
| Festuca arundinacea | 1.21 |
| Festuca gigantea | 1.32 |
| Festuca ovina | 0.592 |
| Festuca pratensis | 1.34 |
| Festuca rubra | 2.68 |
| Glyceria declinata | 0.226 |
| Glyceria fluitans | 0.892 |
| Glyceria maxima | 8.32 |
| Glyceria notata | 0.992 |
| Holcus lanatus | 0.42 |
| Holcus mollis | limit* |
| Hordeum murinum | 0.476 |
| Hordeum secalinum | 0.416 |
| Lolium perenne | 0.452 |
| Milium effusum | 0.524 |
| Molinia caerulea | 2.24 |
| Nardus stricta | 0.246 |
| Phalaris arundinacea | limit* |


| Phleum bertolonii | 0.444 |
| :--- | ---: |
| Phleum pratense | 18 |
| Phragmites australis | 13.2 |
| Poa annua | 0.0844 |
| Poa humilis | 2.37 |
| Poa pratensis | 1.13 |
| Poa trivialis | below detection limit* |
| Puccinellia distans | 11.1 |
| Trisetum flavescens |  |
| Triticum aestivum | 0.736 |
| Vulpia myuros | 1.47 |

[^0]| Expected | rbcL- Control | ITS2- Control | rbcL-Samples | ITS2-Samples |
| :---: | :---: | :---: | :---: | :---: |
| Agrostis |  |  |  | Agrostis |
| Aira |  |  |  |  |
| Alopecurus |  |  |  | Alopecurus |
| Anisantha |  |  |  |  |
| Anthoxanthum <br> Arrhenatherum <br> Avena | Avena | Arrhenatherum | Anthoxanthum <br> Avena | Anthoxanthum <br> Arrhenatherum <br> Avena |
| Brachypodium |  |  |  |  |
| Briza | Briza | Briza/Bromus | Briza |  |
| Bromopsis |  |  |  |  |
| Bromus |  | Briza/Bromus |  |  |
| Catapodium |  |  |  |  |
| Cynosurus Dactylis | Dactylis | Cynosurus Dactylis | Dactylis | Cynosurus |
| Danthonia |  |  |  |  |
| Deschampsia |  | Deschampsia |  | Deschampsia |
| Elymus <br> Elytrigia |  |  |  |  |
| Festuca <br> Glyceria <br> Holcus <br> Hordeum <br> Lolium | Festuca/Lolium | Festuca/Lolium Glyceria <br> Hordeum <br> Lolium | Festuca/Lolium | Festuca/Lolium <br> Holcus <br> Hordeum <br> Lolium |
| Milium |  |  |  |  |
| Molinia | Molinia |  | Molinia |  |
| Nardus Phalaris |  |  |  |  |
| Phleum | Phleum |  | Phleum |  |
| Phragmites |  |  |  |  |
| Poa | Poa | Poa | Poa | Poa |
| Puccinellia <br> Trisetum <br> Triticum <br> Vulpia | Poa |  |  |  |

S2 Table. Genera included in the grass positive control, and genera detected using metabarcoding of both marker genes in both the positive control and in actual aerial DNA extracts. Genera with a grey background were detected by at least one marker gene; genera with a white background were not.

S3 Table. Latitude and longitude of each pollen sampling site.

| Site Name | Abbreviation | Latitude | Longitude |
| :--- | :--- | :--- | :--- |
| Bangor | BNG | 53.2300 | -4.1300 |
| Exeter | EXE | 50.7365 | -3.5322 |
| Invergowrie | ING | 56.4576 | -3.0687 |
| Isle of Wight | IOW | 50.7111 | -1.3009 |
| Worcestershire | WORK | 52.1976 | -2.2430 |
| York | YORK | 53.9484 | -1.0535 |

S4 Table. Sample collection dates of each sequenced air sample. Three consecutive days of air samples were pooled during DNA extraction (note that sample ING_w2_p2, three consecutive samples were unavailable due to sampling error and the next sampling day was selected for pooling).

The mean pollen concentration for the three pooled days and the index i5 and i7 sequence for demultiplexing is shown here.

| Sample | Index i5 and i7 Sequence | Week | Pool | Site | Collection date (2016) | Mean pollen conc. (grains $\mathrm{m}^{-3}$ ) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| BNG_w1_p1 | CAAGTCGT | 1 | 1 | BNG | 25 May - 28 May | 61.7 |
| BNG_w1_p2 | TAACGTCG | 1 | 2 | BNG | 29 May-01 Jun | 27 |
| BNG_w2_p1 | CTGTATGC | 2 | 1 | BNG | 08 Jun - 11 Jun | NA |
| BNG_w2_p2 | TGCTTGCT | 2 | 2 | BNG | 18 Jun - 21 Jun | NA |
| BNG_w3_p1 | GTAGTACC | 3 | 1 | BNG | 24 Jun - 27 Jun | NA |
| BNG_w3_p2 | AAGTCCTC | 3 | 2 | BNG | 27 Jun-30 Jun | NA |
| BNG_w4_p1 | GCATAACG | 4 | 1 | BNG | 08 Jul - 11 Jul | 35.3 |
| BNG_w4_p2 | ATAGTCGG | 4 | 2 | BNG | 11 Jul - 14 Jul | 18.3 |
| BNG_w5_p1 | TAGGAGCT | 5 | 1 | BNG | 21 Jul - 24 Jul | 5.7 |
| BNG_w5_p2 | AGGTGTTG | 5 | 2 | BNG | 25 Jul-28 Jul | 2 |
| BNG_w6_p1 | CATTGACG | 6 | 1 | BNG | 04 Aug - 07 Aug | 4.3 |
| BNG_w6_p2 | CCACAACA | 6 | 2 | BNG | 08 Aug - 11 Aug | 1.3 |
| BNG_w7_p1 | TCTAGGAG | 7 | 1 | BNG | 22 Aug - 25 Aug | 3.3 |
| BNG_w7_p2 | TTGCTTGG | 7 | 2 | BNG | 26 Aug - 29 Aug | 2.3 |
| EXE_w1_p1 | TGATCACG | 1 | 1 | EXE | 02 Jun - 05 Jun | 63 |
| EXE_w1_p2 | TCTGGACA | 1 | 2 | EXE | 06 Jun-09 Jun | 139.3 |
| EXE_w2_p1 | CAGTGCTT | 2 | 1 | EXE | 16 Jun - 19 Jun | 126 |
| EXE_w2_p2 | ATAGGTCC | 2 | 2 | EXE | 20 Jun-23 Jun | 124.7 |
| EXE_w3_p1 | CTGTACCA | 3 | 1 | EXE | 01 Jul - 04 Jul | 52.3 |
| EXE_w3_p2 | AAGCATCG | 3 | 2 | EXE | 04 Jul - 07 Jul | 61.3 |
| EXE_w4_p1 | CCTGTCAA | 4 | 1 | EXE | 14 Jul - 17 Jul | 56 |
| EXE_w4_p2 | AATGGTCG | 4 | 2 | EXE | 17 Jul - 20 Jul | 21.7 |
| EXE_w5_p1 | CTCCTGAA | 5 | 1 | EXE | 29 Jul-01 Aug | 7 |
| EXE_w5_p2 | GACGAACT | 5 | 2 | EXE | 01 Aug - 04 Aug | 2.7 |
| EXE_w6_p1 | GGTCGTAT | 6 | 1 | EXE | 11 Aug - 14 Aug | 2.3 |
| EXE_w6_p2 | AAGTGCAG | 6 | 2 | EXE | 14 Aug - 17 Aug | 3.3 |
| EXE_w7_p1 | CCATGAAC | 7 | 1 | EXE | 25 Aug - 28 Aug | 3 |
| EXE_w7_p2 | TACTAGCG | 7 | 2 | EXE | 28 Aug - 31 Aug | 0.7 |
| ING_w1_p1 | GTGATCCA | 1 | 1 | ING | 30 May - 02 Jun | 2 |
| ING_w1_p2 | ATAACGCC | 1 | 2 | ING | 03 Jun - 06 Jun | 1 |
| ING_w2_p1 | ACCATAGG | 2 | 1 | ING | 13 Jun - 16 Jun | 7 |
| ING_w2_p2 | AGTTCGCA | 2 | 2 | ING | $\begin{aligned} & 16 \text { Jun, } 19 \text { Jun, } \\ & 20 \text { Jun } \end{aligned}$ | 19.3 |
| ING_w3_p1 | CAACTTGG | 3 | 1 | ING | 27 Jun - 30 Jun | 19 |


| ING_w3_p2 | CGCAATGT | 3 | 2 | ING | 30 Jun-03 Jul | 38 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| ING_w4_p1 | GGCTCAAT | 4 | 1 | ING | 18 Jul - 21 Jul | 67.7 |
| ING_w4_p2 | GACTTGTG | 4 | 2 | ING | 21 Jul - 24 Jul | 22.7 |
| ING_w5_p1 | GCTACAAC | 5 | 1 | ING | 25 Jul - 28 Jul | 19.7 |
| ING_w5_p2 | GGTACGAA | 5 | 2 | ING | 28 Jul - 31 Jul | 27.3 |
| ING_w6_p1 | ACGAACGA | 6 | 1 | ING | 09 Aug - 12 Aug | 3.3 |
| ING_w6_p2 | AACACTGG | 6 | 2 | ING | 12 Aug - 15 Aug | 3.3 |
| ING_w7_p1 | TGGATGGT | 7 | 1 | ING | 22 Aug - 25 Aug | 3 |
| IOW_w1_p1 | TACTGCTC | 1 | 1 | IOW | 23 May - 26 May | 7.3 |
| IOW_w1_p2 | CTTCGCAA | 1 | 2 | IOW | 28 May - 31 May | 13.7 |
| IOW_w2_p1 | GATCAAGG | 2 | 1 | IOW | 06 Jun-09 Jun | 253 |
| IOW_w2_p2 | GGCGAATA | 2 | 2 | IOW | 10 Jun-13 Jun | 84 |
| IOW_w3_p1 | CAACGAGT | 3 | 1 | IOW | 19 Jun-22 Jun | 57.7 |
| IOW_w3_p2 | ATCGGAGA | 3 | 2 | IOW | 22 Jun - 25 Jun | 39.7 |
| IOW_w4_p1 | TGTTCCGT | 4 | 1 | IOW | 04 Jul - 07 Jul | 86 |
| IOW_w4_p2 | ATCCACGA | 4 | 2 | IOW | 08 Jul - 11 Jul | 52.3 |
| IOW_w5_p1 | TCACCTAG | 5 | 1 | IOW | 18 Jul-21 Jul | 64.3 |
| IOW_w5_p2 | AGGATAGC | 5 | 2 | IOW | 22 Jul - 25 Jul | 13 |
| IOW_w6_p1 | ATGACAGG | 6 | 1 | IOW | 03 Aug-06 Aug | 5 |
| IOW_w6_p2 | CCGTTATG | 6 | 2 | IOW | 06 Aug - 09 Aug | 6.7 |
| IOW_w7_p1 | ACCTCTTC | 7 | 1 | IOW | 15 Aug - 18 Aug | 4.3 |
| IOW_w7_p2 | ACAGAGGT | 7 | 2 | IOW | 18 Aug - 21 Aug | 2 |
| WOR_w1_p1 | CGCTACAT | 1 | 1 | WOR | 25 May - 28 May | 0 |
| WOR_w1_p2 | AACCAGAG | 1 | 2 | WOR | 29 May-01 Jun | 0 |
| WOR_w2_p1 | GCAATTCC | 2 | 1 | WOR | 08 Jun - 11 Jun | 114.7 |
| WOR_w2_p2 | AGCCGTAA | 2 | 2 | WOR | 11 Jun - 14 Jun | 40.7 |
| WOR_w3_p1 | AACAAGGC | 3 | 1 | WOR | 22 Jun - 25 Jun | 131 |
| WOR_w3_p2 | GAGCAATC | 3 | 2 | WOR | 25 Jun - 28 Jun | 78.7 |
| WOR_w4_p1 | AGTATGCC | 4 | 1 | WOR | 07 Jul - 10 Jul | 76 |
| WOR_w4_p2 | TCGATGAC | 4 | 2 | WOR | 10 Jul - 13 Jul | 16 |
| WOR_w5_p1 | GATACCTG | 5 | 1 | WOR | 20 Jul - 23 Jul | 26.3 |
| WOR_w5_p2 | ACCGACAA | 5 | 2 | WOR | 23 Jul-26 Jul | 16 |
| WOR_w6_p1 | ACGAATCC | 6 | 1 | WOR | 03 Aug - 06 Aug | 0 |
| WOR_w6_p2 | TCGAGAGT | 6 | 2 | WOR | 07 Aug - 10 Aug | 0 |
| WOR_w7_p1 | GTTCTTCG | 7 | 1 | WOR | 17 Aug - 20 Aug | 0 |
| WOR_w7_p2 | CCTTCCAT | 7 | 2 | WOR | 21 Aug - 24 Aug | 0 |
| YORK_w1_p1 | TCCACGTT | 1 | 1 | YORK | 26 May - 29 May | 3 |
| YORK_w1_p2 | TTACCGAC | 1 | 2 | YORK | 29 May-01 Jun | 9.7 |
| YORK_w2_p1 | TTCGCCAT | 2 | 1 | YORK | 08 Jun - 11 Jun | 84.7 |
| YORK_w2_p2 | TATGGCAC | 2 | 2 | YORK | 13 Jun - 16 Jun | 96.7 |
| YORK_w3_p1 | CGCGTATT | 3 | 1 | YORK | 25 Jun - 28 Jun | 178 |
| YORK_w3_p2 | AGCCTATC | 3 | 2 | YORK | 28 Jun-01 Jul | 157 |
| YORK_w4_p1 | GACACAGT | 4 | 1 | YORK | 07 Jul - 10 Jul | 234.3 |


| YORK_w4_p2 | GAGAGTAC | 4 | 2 | YORK | 10 Jul -13 Jul | 245.3 |
| :--- | :--- | :--- | :--- | :--- | :---: | :---: |
| Negative control 1 | CCACTAAG | - | - | - | - | - |
| Negative control 2 | CCACATTG | - | - | - | - | - |
| Negative control 3 | CCGATGTA | - | - | - | - | - |
| Negative control 4 | CTCGGTAA | - | - | - | - | - |
| Negative control 5 | AACCGTGT | - | - | - | - | - |
| Negative control 6 | CGGTTGTT | - | - | - | - | - |
| Negative control 7 | CTAGCAGT | - | - | - | - | - |
| Negative control 8 | ACAACAGC | - | - | - | - | - |
| Negative control 9 | GATTGTCC | - | - | - | - | - |
| Exotic positive control | ACAGGCAT | - | - | - | - | - |
| Grass positive control | TTCGTACG | - | - | - | - | - | demultiplexing samples (see S4 Table for index i5 and i7 sequence).


| Round 1 PCR |
| :---: |
| Forward Universal Tail - NNNNNN - Template Specific Primer rbcLaF <br> [ACACTCTTTCCCTACACGACGCTCTTCCGATCT]-[NNNNNN]-[ATGTCACCACAAACAGAGACTAAAGC] |
| Reverse Universal Tail - Template Specific Primer rbcLr506 <br> [GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT]-[AGGGGACGACCATACTTGTTCA] |
| Forward Universal Tail - NNNNNN - Template Specific Primer ITS2F <br> [ACACTCTTTCCCTACACGACGCTCTTCCGATCT]-[NNNNNN]-[ATGCGATACTTGGTGTGAAT] |
| Reverse Universal Tail - Template Specific Primer ITS3R <br> [GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT]- [GACGCTTCTCCAGACTACAAT] |
| Round 2 PCR |
| P5 Illumina adapter - i5 index - Forward Universal Tail <br> [AATGATACGGCGACCACCGAGATCTACAC]-[ i5 index ]-[ACACTCTTTCCCTACACGACGCTC] |
| P7 Illumina adapter - i7 index - Reverse Universal Tail <br> [CAAGCAGAAGACGGCATACGAGAT]-[i7 index]-[GTGACTGGAGTTCAGACGTGTGCTC] |

S6 Table. Model selection based on AIC criteria. Models shown in bold were used to analyse the data presented here.

| Marker | Model | AIC |
| :---: | :---: | :---: |
| $r b c L$ | (1) Time + Time:Latitude + Latitude + Longitude + Month | 3444.949 |
| $r b c L$ | (2) Time + Time:Latitude + Latitude + Month | 3462.208 |
| $r b c L$ | (3) Time + Time:Latitude + Time:Longitude + Latitude + Longitude + Month | 3467.373 |
| $r b c L$ | (4) Latitude + Month + Longitude + | 3473.788 |
| $r b c L$ | (5) Latitude + Month | 3474.854 |
| $r b c L$ | (6) Time + Time:Longitude + Longitude | 3479.546 |
| $r b c L$ | (7) Time + Time:Latitude + Time:Longitude + Latitude + Longitude + Month500_urban | 3486.374 |
| $r b c L$ | (8) Latitude:Month + Longitude + Month | 3489.559 |
| $r b c L$ | (9) Time + Site_ID + Time:Latitude + Month | 3504.327 |
| $r b c L$ | (10) Time | 3582.784 |
| $r b c L$ | (11) Time + Site_ID + Time:Site_ID + Month | 3584.097 |
| $r b c L$ | (12) Time + Time:Latitude | 3584.776 |
| $r b c L$ | (13) Time + Longitude + | 3586.391 |
| $r b c L$ | (14) Time + Time:Latitude + Time:Longitude + Latitude + Longitude | 3590.813 |
| $r b c L$ | (15) Time + Time:Longitude + Longitude | 3611.398 |
| $r b c L$ | (16) Latitude | 3614.176 |
| $r b c L$ | (17) Time + Site_ID + Time:Site_ID + Latitude + Month | 3615.487 |
| $r b c L$ | (18) Time + Site_ID | 3655.547 |
| $r b c L$ | (19) Site_ID | 3688.586 |
| $r b c L$ | (20) Time + Site_ID + Time:Site ID | 3719.946 |
| $r b c L$ | (21) Time + Site_ID + Time:Site_ID + Latitude | 3751.946 |


| ITS2 | (22) Time + Time:Latitude + Time:Longitude + Latitude + Longitude + Month | 4306.795 |
| :---: | :---: | :---: |
| ITS2 | (23) Time + Time:Latitude + Latitude + Longitude + Month | 4312.207 |
| ITS2 | (24) Time + Time:Latitude + Time:Longitude + Latitude + Longitude + Month +500_urban | 4314.299 |
| ITS2 | (25) Time + Time:Latitude + Latitude + Month | 4328.732 |
| ITS2 | (26) Latitude:Month + Longitude + Month | 4345.029 |
| ITS2 | (27) Latitude + Month + Longitude | 4345.197 |
| ITS2 | (28) Latitude + Month | 4353.331 |
| ITS2 | (29) Time + Site_ID + Time:Latitude + Month | 4362.502 |
| ITS2 | (30) Time + Site_ID + Time:Site_ID + Month | 4363.541 |
| ITS2 | (31) Time + Time:Longitude + Longitude + Month | 4377.7 |
| ITS2 | (32) Time + Site_ID + Time:Site_ID + Latitude + Month | 4392.966 |
| ITS2 | (33) Time + Time:Latitude + Time:Longitude + Latitude + Longitude | 4550.79 |
| ITS2 | (34) Time + Time:Latitude + Latitute | 4569.352 |
| ITS2 | (35) Time + Time:Longitude + Longitude | 4603.281 |
| ITS2 | (36) Time + Longitude | 4604.212 |
| ITS2 | (37) Time | 4610.119 |
| ITS2 | (38) Time + Site_ID | 4625.929 |
| ITS2 | (39) Time + Site_ID + Time:Site ID | 4650.117 |
| ITS2 | (40) Latitude | 4675.797 |
| ITS2 | (41) Time + Site_ID + Time:Site_ID + Latitude | 4680.117 |
| ITS2 | (42) Site_ID | 4747.236 |

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[^0]:    * note these samples successfully amplified using rbcL and ITS2 primers shown in S5 Table.

