Editor (Antonio Amorim)

**Comment:** Line 42: Understanding biodiversity is an important goal of biology and ecology.  
I suggest (since Ecology is a branch of Biology), to keep just: Biology

**Response:** Thank you, this change has been made.  
  
**Comment:** Line 66: These increases are due   
Please contemplate changing the text to: This increase is due  
  
**Response:** Done  
  
**Comment:** Lines 120 – 132: the text written by the authors should not be included in the Material and Methods chapter. Please considering removing or moving it to other chapters (such as Introduction and/or Discussion).  
 **Response:** Thank you for this suggestion, these lines have been moved to the Introduction.  
  
**Comment:** Lines 140 – 143, 198 – 201: the text written by the authors should not be included in the Material and Methods chapter. Please considering removing or moving it to other chapters (such as Introduction and/or Discussion).  
 **Response:** Lines 140-143 have been moved to the Discussion and lines 198-201 have been removed from the manuscript.  
  
**Comment:** Line 569: please include the HPDL acronym in the legend of Table 1.  
 **Response:** Done  
  
**Comment:** I would like the objections 1 to 4 (Rev#2) raised under Experimental design to be answered.

**Response:** Objections have been addressed (*see responses to each specific point below*) and changes in the manuscript as needed.

**Comment:** Typos: Mantel instead of mantel

**Response:** These have been changed.

Reviewer 1 (Anonymous)  
**Comment:** Lines 25 - 26: DNA banks are used as storage repositories for genetic diversity of organisms ranging from plants to insects to mammals throughout the world.  
Please contemplate changing the text to: Throughout the world, DNA banks are used as storage repositories for genetic diversity of organisms ranging from plants to insects to mammals.  
  
**Response:** Done  
  
**Comment:** Lines 27 – 28: These banks preserve the genetic information for organisms of interest, however they also indirectly preserve organisms’ associated microbiomes, including fungi associated with plant tissues.  
Please contemplate changing the text to: Designed to preserve the genetic information for organisms of interest, these banks also indirectly preserve organisms’ associated microbiomes, including fungi living and colonizing plant tissues.  
  
**Response:** Done  
  
**Comment:** Lines 37: please contemplate replacing historic samples by historical samples.  
 **Response:** Done  
  
**Comment:** Line 42: Understanding biodiversity is an important goal of biology and ecology.  
Please contemplate changing the text to: Understanding biodiversity is an important goal of both Biology and Ecology.  
  
**Response:** Thank you we have changed this to Biology as suggested by the editor, since Biology encompasses Ecology.  
  
**Comment:** Line 48: initially developed to collect genetic material in order to create a storage base for evolutionary  
Please contemplate changing the text to: initially developed to collect genetic material to create a storage base for evolutionary.  
 **Response:** Done  
  
**Comment:** Line 55 - 59: These samples represent well-preserved DNA at snapshots in time and from specific locations. For example, plant DNA bank samples not only preserve the targeted species’ genomic information, but also preserve potentially important cryptic microbial symbionts associated with their host, such as fungi known to inhabit the plant phyllosphere (Porras-Alfaro & Bayman, 2011; Vorholt, 2012).  
Please contemplate changing the text to: These samples represent well-preserved DNA at snapshots in time and space. For example, plant DNA bank samples not only preserve the genomic information of the targeted species, but also preserve potentially important cryptic microbial symbionts associated with the host, such as fungi known to inhabit the plant phyllosphere (Porras-Alfaro & Bayman, 2011; Vorholt, 2012).  
  
**Response:** Done  
  
**Comment:** Line 66 - 71: These increases are due in part to advances in direct environmental DNA sequencing and extrapolations based on predictions of vascular plant to fungal ratios (O’Brien et al., 2011; Taylor et al., 2014). In order to obtain more accurate estimates of true fungal diversity, increased sampling using high throughput DNA sequencing of many different types of environments is needed, and DNA banks may significantly contribute to filling this knowledge gap.  
Please contemplate changing the text to: This increase is due, in part, to advances in direct environmental DNA sequencing and extrapolations based on predictions of vascular plant to fungal ratios (O’Brien et al., 2011; Taylor et al., 2014). To obtain more accurate estimates of the true fungal diversity, increased sampling using high throughput DNA sequencing of many different types of environments is needed, and DNA banks may significantly contribute to filling this knowledge gap.  
 **Response:** Done  
  
**Comment:** Line 79: please elucidate how the 86% values was calculated (46/52\*100=88%).  
 **Response:** Thank you for noticing this, this has been changed to 88%.  
  
**Comment:** Lines 92 – 95 and 98 - 101: in the reviewer´s opinion, the text is rather confusing. Please consider re-phrasing it. Also, in line 98, the authors state that all wild plants tissues harbor fungi. In the reviewer´s opinion this is also true for cultivated plants.  
 **Response:** We have re-phrased the text to clarify the confusion. We have also changed all wild plants to all naturally occurring plant tissues.  
  
**Comment:** Lines 101 – 103: Thus, along with its banked plant samples the HPDL has also likely and coincidentally preserved a substantial portion of the diversity of Hawaiian fungi, acting as a repository for not only plant genetics, but their microbiomes as well  
Please contemplate changing the text to: Thus, along with its banked plant samples, the HPDL also has likely and coincidentally preserved a substantial portion of the diversity of Hawaiian fungi, acting as a repository for not only plant genetics, but their microbiomes as well.  
 **Response:** Done  
  
**Comment:** Lines 104: please contemplate replacing historic samples by historical samples.  
 **Response:** Done  
  
**Comment:** Lines 120 – 132: the text written by the authors should not be included in the Material and Methods chapter. Please considering removing or moving it to other chapters (such as Introduction and/or Discussion).  
 **Response:** As also requested by the editor these lines have been moved to the Introduction.  
  
**Comment:** Line 136: please consider removing in the DNA Library.  
 **Response:** Done  
  
**Comment:** Lines 140 – 143, 198 – 201: the text written by the authors should not be included in the Material and Methods chapter. Please considering removing or moving it to other chapters (such as Introduction and/or Discussion).  
 **Response:** As also requested by the editor these changes have been made. Lines 140-143 have been moved to the Discussion and lines 198-201 have been removed from the manuscript.  
  
**Comment:** Lines 215 – 217: in the reviewer´s opinion, the text is rather confusing. Please consider re-phrasing it.  
 **Response:** Changes have been made to these lines to better clarify these methods.  
  
**Comment:** Line 569: please include the HPDL acronym.  
 **Response:** Done

Reviewer 2: (Ricardo Araujo)

**Experimental design**

**Comment:** It is not clarified:  
1) were the plants collected using gloves and protecting the material from hand contamination? how do the authors assured that the plants had similar age, light exposure, similar humidity and environmental conditions? the same plant under different sun exposure for example may hold very distinct microbial communities!

**Response:** Thank you for asking these questions, the plants were not collected using gloves, because they were not collected with microbial sampling in mind, and are therefore not protected from hand contaminants. This has been added to the Methods as well as Discussion sections. However, we feel that the signal to noise ratio is strong enough to outweigh this noise. The plants likely were not at a similar age and environmental conditions and we agree these factors should be taken into consideration in future studies, we have also added this to the Discussion section.

**Comment:** 2) how long were the leaves kept at 4˚C? How long was the DNA stored at -20˚C?

**Response:** We have added this to our Methods section, the leaves were kept at 4˚C for less than a week, generally they were extracted within a few days. The DNA has been stored at -20˚C since extraction dates, which in our study vary from 2 years to our oldest sample collected 10 years prior to our sequencing run. However samples stored in the DNA library span days to over 20 years and were extracted with CsCl banding to obtain stable DNA for long-term storage.

**Comment:** 3) 1g of plant material was extracted per sample. What was the reproducibility comparing samples from the same leave? and from different leaves?

**Response:** The reviewer raises an interesting question about alpha and beta diversity of phyllosphere fungi. However, the goal of this study was not to assess intra- or inter-leaf variability of these fungal communities, but rather assess the diversity of fungi from a single genus of plant from which DNA was extracted and preserved in a similar manner for multiple individuals.

**Comment:** 4) in my opinion UCHIME predicts chimeras much better than any other tool (Perseus or other; no questions about it). But UCHIME2 is much better than UCHIME. Please see documents and publications of Edgar et al. (the developer of the tool). The last version of the tool should be used unless there is a specific reason not to do it (better results with the oldest version for example, but anyway both need to be tested).

**Response:** Thank you for bringing this to our attention, however we have chosen to keep our original analysis using UCHIME because the software’s author, Rober Edgar, does not recommend using the UCHIME2 algorithm for ITS analysis or OTU clustering pipelines. The newer version, UCHIME2, leads to higher false positives, removing good sequences. Edgar, says "It is better to use [unoise](http://www.drive5.com/usearch/manual/cmd_unoise.html) or [cluster\_otus](http://www.drive5.com/usearch/manual/cluster_otus.html) for chimera filtering." We used this "cluster\_otus" approach within the pick\_otus.py workflow within QIIME. This makes use of uparse\_ref internally, which assumes parent sequences to be ANY more abundant (parent abundance >= chimera abundance + 1) rather than at least twice as abundant (parent abundance >= chimera abundance x 2) as in the standard UCHIME and UCHIME2 algorithms, which helps to avoid false positives. The UCHIME2 approach is currently only being recommended for Ribosomal Sequence Variant workflows (i.e., DADA2/Unoise), not clustered OTUs. Further, this newer version is not yet implemented in Vsearch (the open source 'version' of Usearch) which we prefer to use due to cost and transparency. We have added a citation for Usearch into the manuscript to help clarify our methodology.

Please see this Usearch website below for Edgar’s recommendations: <http://www.drive5.com/usearch/manual/uchime2_algo.html>