Title: The fall of the summer truffle: recurring hot, dry summers result in declining fruitbody production of Tuber aestivum in central Europe

Authors: Brian S. Steidinger, Ulf Büntgen, Uli Stobbe, Willy Tegel, Ludger Sproll, Matthias Haeni, Barbara Moser, Istvan Bagi, José-Antonio Bonet, Marc Buée, Benjamin Dauphin, Fernando Martínez-Peña, Virginie Molinier, Roman Zweifel, Simon Egli, Martina Peter

Supplemental Information

Vegetation, Soil Physicochemical, and Soil Fungal Meta-Community Analysis

At 16 of the 20 truffle sites, the vegetation, soil physicochemical and fungal meta-community variables were assessed in a 200 m² circle plot between June and August 2014. The plots centered on the area with the highest T. aestivum production. Percent and total cover of all vascular plant species was estimated in three plant height classes (herb layer 0–50 cm; shrub layer 51–500 cm; tree layer >500 m) as outlined in (Moser et al., 2017) and the percent cover of bare soil (i.e. ground cover) was also estimated. This equaled the soil surface of the circle plot not covered by vascular plants, mosses or litter in the herb layer.

In each of the same 16 sites, soil cores were taken from within the 200 m² vegetation survey area in November 2014. The area was subdivided in 4 quarters, 4 randomly distributed cores (10 cm deep and at least 2 m apart) were taken within each subdivision and pooled. The soil cores were 10 cm deep and 2–3 m separated each core. Soil cores were placed in the cold room (+4°C) for maximum one week before processing. Roots were collected from the 4 soil cores per subdivision, sieved (2 mm), and homogenized. From each homogenate (4 homogenates per monitoring site), three 2 ml tubes were filled for DNA analysis (Illumina) and tubes were frozen at -80°C. The rest of soil was dried for physicochemical analyses for which the four homogenates per monitoring site were pooled and sent to SADEF laboratories (Aspach-le-Bas, France; parameters assessed see Table S1).
Fungal DNA from the soil cores was extracted using the PowerSoil MOBIO DNA Isolation kit according to the manufacturer’s instructions. DNA elution was 10-fold diluted for further analyses. The ITS2 barcode of fungal organisms was amplified using equimolar mixes of the primers ITS3 (Mix1_Fungi, Mix3_Sebacinales, Mix4_Glomeromycota, Mix5_Sordariales) and ITS4 (Mix1_Fungi, Mix4_Tulasnellaceae) as recommended by Tedersoo et al. (2015). Four PCRs per DNA-sample were carried out and pooled (2x51°C, 2x55°C annealing temp). PCR-amplified and AMPPure XP (Beckman Coulter) purified products were sent to Genome Quebec (McGill University) for MiSeq sequencing. Only forward (R1) reads were used (no pairing), since reverse reads showed low quality. In brief, the reads were first filtered and trimmed using the fastq_filter command of USEARCH from UPARSE (Edgar, 2013) and the ITS region sequences were then dereplicated to remove duplicated sequences using the derep_fulllength command of USEARCH. The dereplicated sequences were selected and the singletons were discarded. Operational taxonomic units (OTUs) were generated using USEARCH with a 97% similarity threshold and the taxonomic assignation was determined for each OTU representative sequence using the Basic Local Alignment Search Tool (BLAST) algorithm v 2.2.23 (Altschul et al., 1990) against the UNITE database release (https://unite.ut.ee/). After removing plant reads (the used primers also weakly amplify ITS regions of some plants) and one sample with low read numbers, samples were rarefied to 1,830 reads.

Sequences for each fungal OTU were first aggregated by site (taking the sum over all soil cores). All OTU singletons were removed. Sequences were rarefied to an even depth and converted to proportional abundances for each site. We characterized the orthogonal differences separately for vegetative (tree layer only) and fungal community using three separate principal component (PC) analyses each using the prcomp function in RStudio (v 1.1.463). These fungal and vegetative PC axes were included as potential predictor variables in our machine learning model selection analyses of total fruitbody production among sites and years (Figure S1–2).
Truffle fruiting and tree growth season calculation

To calculate the lengths of the growing and fruiting seasons for host trees and truffles, S-shaped Gompertz functions were fit to the relationship between the numerical day of the year vs tree diameter and cumulative fruitbody production, respectively (Čufar et al., 2008). The Gompertz function is expressed as:

\[ f'(t) + ae^{-be^{(-ct)}} \]  

\text{eq (1)}

Where \( a, b, \) and \( c \) are parameters that describe the maximum (\( a \)) and slope and timing (\( b \) and \( c \)) of tree growth or fruitbody production. These three parameters were fit for each site x year x tree combination (and site x year for truffle fruitbodies) using the least sum of squares method in R (nls function in the base package). The start, end, and point of max growth and fructification were calculated from these fit parameters using linear approximation of the Gompertz function. Accordingly,

Start day:

\[ -\frac{1}{c} \left\{ 1 + \log \left[ \frac{-1}{b} \right] \right\} \]  

\text{eq (2)}

End day:

\[ \frac{1}{c} \left\{ e - 1 - \log \left[ \frac{-1}{b} \right] \right\} \]  

\text{eq (3)}

Max growth rate:

\[ \frac{ae}{e} \]  

\text{eq (4)}

Max growth day:

\[ \frac{1}{c} \left\{ \log \left[ \frac{-1}{b} \right] \right\} \]  

\text{eq (5)}

For host trees, each individual tree was fit a different Gompertz function (for a maximum for 4 per site per year) and treated as a technical replicate. The average start and end days and max-growth-rates of all trees were computed as potential predictors of annual productivity.
Variance Partitioning

For each site x year combination, we summed the annual total number of fruitbodies per unit area (m$^2$ yr$^{-1}$). We then calculated mean truffle mass as the ratio of total annual mass (g yr$^{-1}$) over the total number of truffles (# of truffles yr$^{-1}$). This allowed us to express truffle yield (z, in g m$^2$ yr$^{-1}$) as the product of fruitbody yield (x, in # of fruitbodies m$^2$ yr$^{-1}$) and mean truffle mass (y, in g). In order to correct for the differences in scale between the variables x and y (measured in different units with multiplicative effects on z), we log-transformed the product $z = x \times y$ into the sum $\log(z) = \log(x) + \log(y)$. This allowed us to partition the variance in $\log(z)$ into the sum of two covariance terms (for x and y), such that

$$\text{var}[\log(z)] = \text{cov}[\log(x), \log(z)] + \text{cov}[\log(y), \log(z)].$$

When both covariance terms are positive (as they are in our analysis), then years with a greater truffle yield (z) tend to have both a greater number of truffles (x) and a greater mean truffle mass (y). This means that the ratio of the x and y covariance terms over var[log(z)] gives the proportion of the variance accounted for by x (truffle numbers) and y (mean truffle mass), respectively.

Variables and Model Selection

A large suite of predictors was assembled and exposed to model selection using the random forest algorithm. These were plotted in Figure 4a. We provide a full list of these variables along with the units and data source in Table S1.

Principal Component Analyses

For vegetative and soil meta-community variables principle component analyses (package “prcomp” in R) were performed to collapse the high dimensional datasets into three orthogonal axes (Supplemental Figures S1,S2).

For soil fungal meta-communities, there were 2,219 OTUs (after rarefaction and removal of singletons). This makes visualizing loading values with vectors on top of the PCA scatterplots difficult (particularly when points are annotated by site), due to the overlapping
labels. In Figure S2 (top row) we characterized the top 5 positive and negative loading values on the first 3 PCA axes.

For the dendrometer data, we conducted a separate principal component analysis. We included data for temperature and precipitation during the hottest quarter, mean tree growth during the hottest quarter, and mean tree water deficit during the warmest quarter (Figure 6a).
Supplemental Table 1. All variables used in the random forest analysis of annual truffle yield (g m\(^{-2}\) yr\(^{-1}\)). Variables are sorted according to the rank of their Inc Node Purity importance scores (as in the x-axis of Figure 3). The %MSE column gives another metric of variable importance (along with the numerical rank).

| rank | variable                                                | source   | variable type | IncNod   | %MSE (rank) |
|------|---------------------------------------------------------|----------|---------------|----------|-------------|
| 1    | pH (in water)                                           | in situ  | soil          | 8424.14  | 12.11 (1)   |
| 2    | bare soil (%)                                           | in situ  | ground cover  | 5334.86  | 10.79 (2)   |
| 3    | precipitation sum of hottest quarter (mm)               | ERA5     | annual climate| 3121.92  | 5.22 (4)    |
| 4    | shrub cover (%)                                         | in situ  | vegetation    | 2405.20  | 7.43 (3)    |
| 5    | mean temperature hottest quarter (°C)                   | ERA5     | annual climate| 1890.10  | 2.73 (14.5) |
| 6    | number of years harvested (yr)                          | instrumental | sampling | 1827.13  | 0.21 (60)   |
| 7    | herb cover (%)                                          | in situ  | vegetation    | 1688.63  | 4.71 (5)    |
| 8    | soil CaCO\(_3\) (ppm)                                  | in situ  | soil          | 1306.72  | 4.45 (6)    |
| 9    | samples (# of surveys yr\(^{-1}\))                      | Instrumental | sampling | 993.73   | -1.90 (63)  |
| 10   | litter cover (%)                                        | in situ  | ground cover  | 661.81   | 3.94 (8)    |
| 11   | precipitation sum of wettest month (mm)                 | WorldClim | climate means | 354.73   | 1.89 (31)   |
| 12   | total nitrogen (ppm)                                    | in situ  | soil          | 341.11   | 2.49 (18)   |
| 13   | mean diurnal temperature range                          | WorldClim | climate means | 332.22   | 2.73 (14.5) |
| 14   | total annual precipitation (mm)                         | WorldClim | climate means | 320.94   | 2.72 (16)   |
| 15   | coarse sand (ppm)                                       | in situ  | soil          | 311.33   | 2.86 (11.5) |
| 16   | precipitation sum of wettest quarter (mm)               | WorldClim | climate means | 293.82   | 3.13 (10)   |
| 17   | exchangeable CaO (ppm)                                  | in situ  | soil          | 275.27   | 3.35 (9)    |
| 18   | tree composition PC2                                    | in situ  | vegetation    | 274.44   | 2.59 (17)   |
| 19   | organic carbon (ppm)                                    | in situ  | soil          | 254.13   | 2.32 (21)   |
| 20   | mean min temperature of coldest month (°C)              | WorldClim | climate means | 222.87   | 4.05 (7)    |
| 21   | shrub layer species richness (# of sp)                  | in situ  | vegetation    | 221.96   | 2.86 (11)   |
|   | Description                                                                 | Source                        | Value     | SE     | N   |
|---|------------------------------------------------------------------------------|-------------------------------|-----------|--------|-----|
| 22| fine sand (ppm)                                                              | in situ soil                  | 188.65    | 1.86   | 32  |
| 23| elevation (m a.s.l)                                                          | in situ elevation             | 184.95    | 2.44   | 19  |
| 24| moss cover (%)                                                               | in situ vegetation            | 176.34    | 2.34   | 20  |
| 25| mean temperature of wettest quarter (°C)                                     | WorldClim climate means      | 172.42    | 1.40   | 51  |
| 26| C:N ratio                                                                    | in situ soil                  | 167.43    | 2.28   | 22  |
| 27| precipitation seasonality (c.o.v)                                           | WorldClim climate means      | 161.88    | 1.38   | 52  |
| 28| precipitation sum of driest quarter (mm)                                     | WorldClim climate means      | 157.85    | 1.44   | 49  |
| 29| soil fungi composition PC3                                                   | in situ fungal community     | 140.07    | 2.07   | 25  |
| 30| organic matter (ppm)                                                         | in situ soil                  | 134.56    | 1.80   | 35  |
| 31| precipitation sum of warmest quarter (mm)                                    | WorldClim climate means      | 129.60    | 1.95   | 28  |
| 32| soil fungi composition PC2                                                   | in situ fungal community     | 128.51    | 1.83   | 33  |
| 33| herb species richness (# of sp)                                              | in situ vegetation            | 128.29    | 1.79   | 36  |
| 34| soil P2O5 (Joret-Hebert extraction, ppm)                                     | in situ soil                  | 125.21    | 1.56   | 43  |
| 35| plant species richness (# of sp)                                             | in situ vegetation            | 124.06    | 2.06   | 26  |
| 36| mean annual soil temperature (°C)                                            | WorldClim climate means      | 121.93    | 2.08   | 24  |
| 37| cation exchange capacity (meq)                                                | in situ soil                  | 118.41    | 2.00   | 27  |
| 38| mean temperature of coldest quarter (°C)                                     | WorldClim climate means      | 115.96    | 1.93   | 29.5|
| 39| exchangeable K2O (ppm)                                                       | in situ soil                  | 105.39    | 1.78   | 37  |
| 40| soil fungi composition PC1                                                   | in situ fungal community     | 104.25    | 1.54   | 44  |
| 41| clay (ppm)                                                                   | in situ soil                  | 103.08    | 1.93   | 29.5|
| 42| isothermality                                                                 | WorldClim climate means      | 99.69     | 1.81   | 34  |
| 43| tree / shrub layer species richness (# of sp)                                | in situ vegetation            | 93.93     | 0.36   | 59  |
| 44| temperature seasonality (std x 100)                                           | WorldClim climate means      | 92.02     | 2.21   | 23  |
| 45| precipitation sum of driest month (mm)                                       | WorldClim climate means      | 89.92     | 1.52   | 46  |
| 46| mean temperature of the hottest quarter                                      | ERA5 climate means           | 89.44     | 1.35   | 54  |
| 47| tree composition PC1                                                         | in situ vegetation            | 81.89     | 1.25   | 56  |
| 48| soil NO3-N (ppm)                                                             | in situ soil                  | 80.51     | 2.77   | 13  |
|   | Parameter Description                                      | Source                  | Measurement Method | Mean    | Std. Dev (N) |
|---|----------------------------------------------------------------|-------------------------|--------------------|---------|--------------|
| 49| mean July soil temperature (°C)                              | WorldClim               | climate means      | 75.54   | 1.76 (38)    |
| 50| precipitation sum of coldest quarter (mm)                   | WorldClim               | climate means      | 70.06   | 1.41 (50)    |
| 51| tree species richness (# of sp)                             | in situ                 | vegetation         | 69.94   | 1.47 (48)    |
| 52| coarse silt (ppm)                                           | in situ                 | soil               | 58.39   | 1.62 (41)    |
| 53| mean max temperature of hottest month (°C)                  | WorldClim               | climate means      | 45.58   | 1.52 (46)    |
| 54| fine silt (ppm)                                             | in situ                 | soil               | 44.84   | 1.68 (39)    |
| 55| tree composition PC3                                        | in situ                 | vegetation         | 43.28   | 1.34 (55)    |
| 56| mean temperature of warmest quarter (°C)                    | WorldClim               | climate means      | 42.29   | 1.14 (58)    |
| 57| NH4 - N on dry soil (ppm)                                   | in situ                 | soil               | 32.02   | -0.91 (62)   |
| 58| mean temperature of driest quarter (°C)                     | WorldClim               | climate means      | 31.69   | -0.06 (61)   |
| 59| soil exchangeable MgO (ppm)                                 | in situ                 | soil               | 13.26   | 1.66 (40)    |
| 60| tree layer (%)                                              | in situ                 | vegetation         | 12.25   | 1.15 (57)    |
| 61| mean annual temperature (°C)                                | WorldClim               | climate means      | 11.34   | 1.61 (42)    |
| 62| mean annual temperature range (°C)                          | WorldClim               | climate means      | 8.51    | 1.52 (46)    |
| 63| active soil CaCO3 (ppm)                                     | in situ                 | soil               | 0.70    | 1.36 (53)    |
Supplemental Figures

Figure S1. Pairwise scatterplots for all combinations of the first three principal components for soil fungal meta-community (top row) and tree species composition (bottom row). The % variability explained by each PC axis is listed parenthetically. Points are annotated by site name.

Figure S2. The top 5 positive and top 5 negative loading values for soil fungal meta-communities (top) and tree species composition (bottom) for the three PC axes.
Figure S3. Temperature and precipitation during the hottest quarter are negatively correlated. Sites are annotated by year X (201X).

Figure S4. The log-transformed annual total number of truffles surveyed vs the total annual truffle mass with a linear best fit line (p<0.001, $R^2 = 0.89$). Points are colored according to the log-transformed mean mass of truffles.
Bibliography

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Čufar, K., Prislan, P., de Luis, M., & Gričar, J. (2008). Tree-ring variation, wood formation and phenology of beech (Fagus sylvatica) from a representative site in Slovenia, SE Central Europe. *Trees, 22*(6), 749–758. https://doi.org/10.1007/s00468-008-0235-6

Edgar, R. C. (2013). UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nature Methods, 10*(10), 996–998. https://doi.org/10.1038/nmeth.2604

Moser, B., Büntgen, U., Molinier, V., Peter, M., Sproll, L., Stobbe, U., Tegel, W., & Egli, S. (2017). Ecological indicators of Tuber aestivum habitats in temperate European beech forests. *Fungal Ecology, 29*, 59–66. https://doi.org/10.1016/j.funeco.2017.06.002