# Supplementary Material



Figure S1. Reconstructed petri dish of NaClO 12.5% at the final time step (T4) and automated identification of charcoal fragments in ImageJ.



Figure S2. Charcoal number of fragments change over 12h and 6h time steps when subjected to widely used chemicals in charcoal analysis. Samples constitute a known mixture of modern charcoal types produced in the lab. For every chemical treatment and two H2O control sets n = 10. Mixed ANOVAs were not possible for this data set due to violations on the assumption of sphericity. Color boxes are the same used as in Figures 2 and 3 to indicate the same treatments.

Table S1: Chemicals, concentrations, and exposure times used for the extraction of charcoal for fire studies around the world. More than one chemical usually indicates a second bleaching step. For full names of the chemicals see text. n/a = not available information in the referenced publication. Note this is not an exhaustive list. Studies were chosen on the basis that they were from all around the world and using different chemicals or combinations thereof.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Reference | Chemical | Conc. | Time | Sediment type | Country | Notes |
| Blarquez et al., 2018 | NaClO | 8% | 24 h | Peat | Canada |  |
| Hawthorne and Mitchell, 2016 | (NaPO3)6H2O2 | 15%diluted | 24 hn/a | Lacustrine | Ireland |  |
| Crawford and Belcher, 2016 | NaClO | 8% | 20 h | Peat | UK |  |
| Fletcher et al., 2018 | NaClO | n/a | n/a | Lacustrine | Tasmania |  |
| Mariani and Fletcher, 2017 | NaClO | 5% | <2 weeks | Multiple | Australia |  |
| Walsh et al., 2017 | (NaPO3)6NaClO | 5%weak | >24 h1h | Lacustrine | USA |  |
| Finsinger et al., 2017 | NaOHNaClO | 10%2% | n/an/a | Lacustrine | Romania |  |
| Anderson and Wahl, 2016 | (NaPO3)6NaClO | 3%6% | >24 hn/a | Lacustrine | Guatemala |  |
| Pérez -Obiol et al., 2016 | KOHNaClO | n/a15% | 1.5 h1.5 h | Peat | Spain | samples heated to 70°C |
| Chipman et al., 2015 | NaO3PNaClO | 10%n/a | 20h20h | Lacustrine | Alaska, USA | freeze-dried overnight / chemicals possibly mixed |
| Robin et al., 2013 | NaClO | 13% | 24 h | Peat | Germany |  |
| Genries et al., 2012 | NaP2O4NaClO | 3%10% | n/an/a | Lacustrine | Canada |  |
| Olsson et al., 2010 | NaClO | 5% | 24 h | Lacustrine | Sweden |  |
| Gardner and Whitlock, 2001 | (NaPO3)6NaClO | 1%5% | 24 h5min | Lacustrine | Northwest USA | heated at low temperature to remove excessive invertebrate faeces |
| Glais et al., 2017 | NaOHH2O2 | 10%6% | 24 h24 h | Peat | Greece |  |
| Finsinger et al., 2017 | H2O2 | 15% | overnight | Peat | Serbia |  |
| Spencer et al., 2017 | H2O2 | 3% | n/a | Lacustrine | USA |  |
| Pillai et al., 2017 | NaO3P H2O2 | 5%8% | n/a12h | wetland cores | India |  |
| Colombaroli et al., 2018 | NaO3P H2O2 | 5%8% | n/a12h | Lacustrine | Kenya |  |
| Rius et al., 2011 | NaOHH2O2 | 10%6% | 24 h24 h | Multiple | France |  |
| Mustaphi and Pisaric, 2014 | NaO3P |  n/a | 24 h | Lacustrine | Canada |  |
| Miyabuchi et al., 2012 | (NaPO3)6KOHH2O2 | 5%10%1% | 24 h24 hn/a | Tephra | Japan |  |
| Olsson et al., 2010 | NaOH | 10% | 24 h | Lacustrine | Sweden |  |
| Thevenon et al., 2003 | HCLHNO3H2O2 | 3Mconc.33% |  n/a n/a n/a | Lacustrine | Tanzania |  |

Table S2: Mean and standard deviation of normalized replicates within each time step for the 12 h batch. Results of repeated measures ANOVA for the 12h batch—significance of the effect of treatment noted.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Overall | **H2O2 33%** | *2.05±1.19* |  |  |  |  |
|  | **H2O2 8%** | 0.99±0.57 | 0.99±0.57 |  |  |  |
|  | **HNO3 50%** | 1.38±0.81 | 1.38±0.81 | 1.38±0.81 |  |  |
|  | **KOH 10%** | 1.65±1.08 | 1.65±1.08 | 1.65±1.08 | 1.65±1.08 |  |
|  | **(NaPO3)6 20%** | *1.95±1.11* | 1.95±1.11 | 1.95±1.11 | 1.95±1.11 | 1.95±1.11 |
| T0 | **H2O2 33%** | 0±1 |  |  |  |  |
|  | **H2O2 8%** | 0±1 | 0±1 |  |  |  |
|  | **HNO3 50%** | 0±1 | 0±1 | 0±1 |  |  |
|  | **KOH 10%** | 0±1 | 0±1 | 0±1 | 0±1 |  |
|  | **(NaPO3)6 20%** | 0±1 | 0±1 | 0±1 | 0±1 | 0±1 |
| T1 | **H2O2 33%** | 2.01±1.06 |  |  |  |  |
|  | **H2O2 8%** | 1.05±0.71 | 1.05±0.71 |  |  |  |
|  | **HNO3 50%** | 1.42±0.82 | 1.42±0.82 | 1.42±0.82 |  |  |
|  | **KOH 10%** | 1.17±1.22 | 1.17±1.22 | 1.17±1.22 | 1.17±1.22 |  |
|  | **(NaPO3)6 20%** | 2.14±1.14 | 2.14±1.14 | 2.14±1.14 | 2.14±1.14 | 2.14±1.14 |
| T2 | **H2O2 33%** | ***2.79±0.78*** |  |  |  |  |
|  | **H2O2 8%** | 1.43±0.58 | 1.43±0.58 |  |  |  |
|  | **HNO3 50%** | 1.60±0.80 | 1.60±0.80 | 1.60±0.80 |  |  |
|  | **KOH 10%** | 2.00±1.20 | 2.00±1.20 | 2.00±1.20 | 2.00±1.20 |  |
|  | **(NaPO3)6 20%** | *2.68±0.92* | 2.68±0.92 | 2.68±0.92 | 2.68±0.92 | 2.68±0.92 |
| T3 | **H2O2 33%** | ***2.62±0.69*** |  |  |  |  |
|  | **H2O2 8%** | 1.26±0.63 | 1.26±0.63 |  |  |  |
|  | **HNO3 50%** | 1.85±0.86 | 1.85±0.86 | 1.85±0.86 |  |  |
|  | **KOH 10%** | ***2.39±1.14*** | 2.39±1.14 | 2.39±1.14 | 2.39±1.14 |  |
|  | **(NaPO3)6 20%** | *2.56±0.98* | 2.56±0.98 | 2.56±0.98 | 2.56±0.98 | 2.56±0.98 |
| T4 | **H2O2 33%** | ***2.81±0.64*** |  |  |  |  |
|  | **H2O2 8%** | 1.21±0.42 | **1.21±0.42** |  |  |  |
|  | **HNO3 50%** | 2.04±0.82 | 2.04±0.82 | *2.04±0.82* |  |  |
|  | **KOH 10%** | *2.67±1.29* | 2.67±1.29 | 2.67±1.29 | 2.67±1.29 |  |
|  | **(NaPO3)6 20%** | 2.39±1.29 | 2.39±1.29 | 2.39±1.29 | 2.39±1.29 | 2.39±1.29 |
|  |  | **H2O 12h** | **H2O2 33%** | **H2O2 8%** | **HNO3 50%** | **KOH 10%** |

p>0.5; *p<0.05*; ***p<0.01***; **p<0.001**

Table S3: Mean and standard deviation of normalized replicates within each time step for the 6 h batch. Results of repeated measures ANOVA for the 6h batch—significance of the effect of treatment noted.

|  |  |  |  |
| --- | --- | --- | --- |
| Overall | **NaClO 2.5%** | **0.06±0.62** |  |
|  | **H2O** | **0.24±0.26** | 0.24±0.26 |
| T0 | **NaClO 2.5%** | 0±1 |  |
|  | **H2O** | 0±1 | 0±1 |
| T1 | **NaClO 2.5%** | **0.920±0.42** |  |
|  | **H2O** | **0.500±0.54** | 0.500±0.54 |
| T2 | **NaClO 2.5%** | **0.29±0.66** |  |
|  | **H2O** | ***−0.66±1.13*** | −0.66±1.13 |
| T3 | **NaClO 2.5%** | **−0.12*±0.62*** |  |
|  | **H2O** | **0.47*±1.36*** | 0.47*±1.36* |
| T4 | **NaClO 2.5%** | **−0.79±0.47** |  |
|  | **H2O** | **0.30±0.68** | ***0.30±0.68*** |
|  |  | **NaClO 12.5%** | **NaClO 2.5%** |

p>0.5; *p<0.05*; ***p<0.01***; **p<0.001**

Table S4: Mean and standard deviation of normalized replicates within each chemical for the 12 h batch. Results of repeated measures ANOVA for the 12 h batch—significance of the effect of time noted.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Overall | **T1** | **1.44±0.22** |  |  |  |
|  | **T2** | **1.92±0.21** | **1.92±0.21** |  |  |
|  | **T3** | **1.92±0.19** | **1.92±0.19** | 1.92±0.19 |  |
|  | **T4** | **1.20±0.35** | **1.20±0.35** | 1.20±0.35 | 1.20±0.35 |
| H2O 12h | **T1** | *0.85±0.73* |  |  |  |
|  | **T2** | ***1.05±0.91*** | 1.05±0.91 |  |  |
|  | **T3** | 0.84±0.82 | 0.84±0.82 | 0.84±0.82 |  |
|  | **T4** | *0.88±0.77* | 0.88±0.77 | 0.88±0.77 | 0.88±0.77 |
| H2O2 33% | **T1** | **2.01±1.06** |  |  |  |
|  | **T2** | **2.79±0.78** | *2.79±0.78* |  |  |
|  | **T3** | **2.62±0.69** | 2.62±0.69 | 2.62±0.69 |  |
|  | **T4** | **2.81±0.64** | 2.81±0.64 | 2.81±0.64 | 2.81±0.64 |
| H2O2 8% | **T1** | *1.05±0.71* |  |  |  |
|  | **T2** | **1.43±0.57** | 1.43±0.57 |  |  |
|  | **T3** | **1.26±0.63** | 1.26±0.63 | 1.26±0.63 |  |
|  | **T4** | *1.21±0.42* | 1.21±0.42 | 1.21±0.42 | 1.21±0.42 |
| HNO3 50% | **T1** | **1.42±0.82** |  |  |  |
|  | **T2** | **1.60±0.80** | 1.60±0.80 |  |  |
|  | **T3** | **1.85±0.86** | *1.85±0.86* | 1.85±0.86 |  |
|  | **T4** | **2.04±0.82** | ***2.04±0.82*** | *2.04±0.82* | *2.04±0.82* |
| KOH 10% | **T1** | 1.17*±1.22* |  |  |  |
|  | **T2** | ***2.00±1.20*** | 2.00±1.20 |  |  |
|  | **T3** | **2.39±1.14** | ***2.39±1.14*** | 2.39±1.14 |  |
|  | **T4** | **2.67±1.29** | **2.67±1.29** | *2.67±1.29* | 2.67±1.29 |
| (NaPO3)620% | **T1** | ***2.14±1.14*** |  |  |  |
|  | **T2** | **2.68±0.92** | 2.68±0.92 |  |  |
|  | **T3** | ***2.56±0.98*** | 2.56±0.98 | 2.56±0.98 |  |
|  | **T4** | ***2.38±1.29*** | 2.38±1.29 | 2.38±1.29 | 2.38±1.29 |
|  |  | **T0** | **T1** | **T2** | **T3** |

p>0.5; *p<0.05*; ***p<0.01***; **p<0.001**

Table S5: Mean and standard deviation of normalized replicates within each chemical for the 6 h batch. Results of repeated measures ANOVA for the 6h batch—significance of the effect of time noted.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Overall | **T1** | 0.28±0.06 |  |  |  |
|  | **T2** | −0.48±0.35 | **−0.48±0.35** |  |  |
|  | **T3** | −0.53±0.53 | **−0.53±0.53** | −0.53±0.53 |  |
|  | **T4** | **−0.83±0.13** | **−0.83±0.13** | *−0.83±0.13* | *−0.83±0.13* |
| NaClO 12.5% | **T1** | −0.59±0.45 |  |  |  |
|  | **T2** | ***−1.65±0.45*** | **−1.65±0.45** |  |  |
|  | **T3** | **−1.94±0.33** | **−1.94±0.33** | *−1.94±0.33* |  |
|  | **T4** | **−2.18±0.44** | **−2.18±0.44** | −2.18±0.44 | −2.18±0.44 |
| NaClO 2.5% | **T1** | 0.92±0.42 |  |  |  |
|  | **T2** | 0.29±0.66 | *0.29±0.66* |  |  |
|  | **T3** | −0.11±0.62 | **−0.11±0.62** | −0.11±0.62 |  |
|  | **T4** | 0.30±0.68 | **0.30±0.68** | **0.30±0.68** | ***0.30±0.68*** |
| H2O 6h | **T1** | 0.50±0.54 |  |  |  |
|  | **T2** | −0.67±1.13 | −0.67±1.13 |  |  |
|  | **T3** | 0.47±1.36 | 0.47±1.36 | 0.47±1.36 |  |
|  | **T4** | 0.30±0.68 | 0.30±0.68 | 0.30±0.68 | 0.30±0.68 |
|  |  | **T0** | **T1** | **T2** | **T3** |

p>0.5; *p<0.05*; ***p<0.01***; **p<0.001**

Table S6: Spearman Correlation coefficients between sequences analysed with the same chemicals.

|  |  |  |
| --- | --- | --- |
| Chemical Treatment | Sequences | Spearman Correlation |
| H2O2 33% | A vs D | 0.223 |
| A vs G | 0.549\* |
| D vs G | 0.517\* |
| NaClO 12.5% | B vs E | 0.512 |
| B vs H | 0.653\* |
| E vs H | 0.741\*\* |
| HNO3 50% | C vs F | 0.824\*\* |
| C vs I | 0.591\* |
| F vs I | 0.685\*\* |

\*\*Correlation significant at the 0.01 level \*Correlation significant at the 0.05 level

Table S7: Spearman Correlation coefficients between numbers (# cm¯³) and area (mm³ cm¯³) of fragments for the nine fossil sequences. Chemical treatment used to analyse each sequence also given.

|  |  |  |
| --- | --- | --- |
|  | Treatment | Spearman Correlation |
| SeqA | H2O2 33% | 0.898\*\* |
| SeqB | NaClO 12.5% | 0.897\*\* |
| SeqC | HNO3 50% | 0.937\*\* |
| SeqD | H2O2 33% | 0.951\*\* |
| SeqE | NaClO 12.5% | 0.833\*\* |
| SeqF | HNO3 50% | 0.923\*\* |
| SeqG | H2O2 33% | 0.936\*\* |
| SeqH | NaClO 12.5% | 0.916\*\* |
| SeqI | HNO3 50% | 0.962\*\* |

\*\*Correlation significant at the 0.01 level

REFERENCES

Anderson, L., & Wahl, D., 2016. Two Holocene paleofire records from Peten, Guatemala: Implications for natural fire regime and prehispanic Maya land use. *Global and Planetary Change*, *138*, 82-92. <https://doi.org/10.1016/j.gloplacha.2015.09.0123>

Blarquez, O., Talbot, J., Paillard, J., Lapointe-Elmrabti, L., Pelletier, N., St-Pierre, C.G., 2018. Late Holocene influence of societies on the fire regime in southern Québec temperate forests. *Quaternary Science Reviews*, *180*, 63-74.<https://doi.org/10.1016/j.quascirev.2017.11.022>

Chipman, M.L., Hudspith, V., Higuera, P.E., Duffy, P.A., Kelly, R., Oswald, W.W., Hu, F.S., 2015. Spatiotemporal patterns of tundra fires: late-Quaternary charcoal records from Alaska. *Biogeosciences*, *12*(13), 4017-4027. doi:10.5194/bg-12-4017-2015

Colombaroli, D., van der Plas, G., Rucina, S., Verschuren, D., 2018. Determinants of savanna-fire dynamics in the eastern Lake Victoria catchment (western Kenya) during the last 1200 years. *Quaternary International*, *488*, 67-80. <https://doi.org/10.1016/j.quaint.2016.06.028>

Crawford, A.J., & Belcher, C.M., 2016. Area–volume relationships for fossil charcoal and their relevance for fire history reconstruction. *The Holocene*, *26*(5), 822-826. <https://doi.org/10.1177/0959683615618264>

Finsinger, W., Morales-Molino, C., Gałka, M., Valsecchi, V., Bojovic, S., Tinner, W., 2017. Holocene vegetation and fire dynamics at Crveni Potok, a small mire in the Dinaric Alps (Tara National Park, Serbia). *Quaternary science reviews*, *167*, 63-77. <https://doi.org/10.1016/j.quascirev.2017.04.032>

Fletcher, M.S., Bowman, D.M.J.S., Whitlock, C., Mariani, M., Stahle, L., 2018. The changing role of fire in conifer-dominated temperate rainforest through the last 14,000 years. *Quaternary Science Reviews*, *182*, 37-47. <https://doi.org/10.1016/j.quascirev.2017.12.023>

Gardner, J.J., Whitlock, C., 2001. Charcoal accumulation following a recent fire in the Cascade Range, northwestern USA, and its relevance for fire-history studies. *The Holocene*, *11*(5), 541-549. <https://doi.org/10.1191/095968301680223495>

Genries, A., Finsinger, W., Asnong, H., Bergeron, Y., Carcaillet, C., Garneau, M., et al., 2012. Local versus regional processes: can soil characteristics overcome climate and fire regimes by modifying vegetation trajectories?. *Journal of Quaternary Science*, *27*(7), 745-756.  [**https://doi.org/10.1002/jqs.2560**](https://doi.org/10.1002/jqs.2560)

Glais, A., Lespez, L., Vannière, B., Lopez-Saez, J.A., 2017. Human-shaped landscape history in NE Greece. A palaeoenvironmental perspective. *Journal of Archaeological Science: Reports*, *15*, 405-422. <https://doi.org/10.1016/j.jasrep.2017.06.017>

Hawthorne, D., & Mitchell, F J., 2016. Identifying past fire regimes throughout the Holocene in Ireland using new and established methods of charcoal analysis. *Quaternary Science Reviews*, *137*, 45-53. <https://doi.org/10.1016/j.quascirev.2016.01.027>

Mariani, M., Fletcher, M.S., 2017. Long-term climate dynamics in the extra-tropics of the South Pacific revealed from sedimentary charcoal analysis. *Quaternary Science Reviews*, *173*, 181-192. <https://doi.org/10.1016/j.quascirev.2017.08.007>

Miyabuchi, Y., Sugiyama, S., Nagaoka, Y., 2012. Vegetation and fire history during the last 30,000 years based on phytolith and macroscopic charcoal records in the eastern and western areas of Aso Volcano, Japan. *Quaternary International*, *254*, 28-35. <https://doi.org/10.1016/j.quaint.2010.11.019>

Mustaphi, C.J.C., Pisaric, M.F., 2014. Holocene climate–fire–vegetation interactions at a subalpine watershed in southeastern British Columbia, Canada. *Quaternary Research*, *81*(2), 228-239. <https://doi.org/10.1016/j.yqres.2013.12.002>

Olsson, F., Gaillard, M.J., Lemdahl, G., Greisman, A., Lanos, P., Marguerie, D., et al., 2010. A continuous record of fire covering the last 10,500 calendar years from southern Sweden—The role of climate and human activities. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *291*(1-2), 128-141. <https://doi.org/10.1016/j.palaeo.2009.07.013>

Pérez-Obiol, R., García-Codron, J.C., Pelachs, A., Pérez-Haase, A., Soriano, J.M., 2016. Landscape dynamics and fire activity since 6740 cal yr BP in the Cantabrian region (La Molina peat bog, Puente Viesgo, Spain). *Quaternary Science Reviews*, *135*, 65-78.

Pillai, A.A., Anoop, A., Sankaran, M., Sanyal, P., Jha, D.K., Ratnam, J., 2017. Mid-late Holocene vegetation response to climatic drivers and biotic disturbances in the Banni grasslands of western India. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *485*, 869-878. <https://doi.org/10.1016/j.palaeo.2017.07.036>

Rius, D., Vannière, B., Galop, D., Richard, H., 2011. Holocene fire regime changes from multiple-site sedimentary charcoal analyses in the Lourdes basin (Pyrenees, France). *Quaternary Science Reviews*, *30*(13-14), 1696-1709. <https://doi.org/10.1016/j.quascirev.2011.03.014>

Robin, V., Knapp, H., Bork, H.R., & Nelle, O., 2013. Complementary use of pedoanthracology and peat macro-charcoal analysis for fire history assessment: illustration from Central Germany. *Quaternary International*, *289*, 78-87. <https://doi.org/10.1016/j.quaint.2012.03.031>

Spencer, J., Jones, K.B., Gamble, D.W., Benedetti, M.M., Taylor, A.K., Lane, C.S., 2017. Late-Quaternary records of vegetation and fire in southeastern North Carolina from Jones Lake and Singletary Lake. *Quaternary Science Reviews*, *174*, 33-53. <https://doi.org/10.1016/j.quascirev.2017.09.001>

Thevenon, F., Williamson, D., Vincens, A., Taieb, M., Merdaci, O., Decobert, M., Buchet, G., 2003. A late-Holocene charcoal record from Lake Masoko, SW Tanzania: climatic and anthropologic implications. *The Holocene*, *13*(5), 785-792. <https://doi.org/10.1191/0959683603hl665rr>

Walsh, M.K., Lukens, M.L., McCutcheon, P.T., Burtchard, G.C., 2017. Fire-climate-human interactions during the postglacial period at Sunrise Ridge, Mount Rainier National Park, Washington (USA). *Quaternary Science Reviews*, *177*, 246-264. <https://doi.org/10.1016/j.quascirev.2017.10.032>