

Headspace volatile organic compounds from bacteria implicated in ventilator-associated pneumonia

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Supplementary information

Growth curves

Growth curves of several *E. coli* knockout (KO) and wildtype (WT) strains in minimal medium are presented in Fig S1. Individual genes postulated to be involved in tryptophan breakdown to produce indole have been knocked out in the KO strains. These include: the *tnaA* gene which encodes the enzyme tryptophanase that catalyses tryptophan breakdown; *tnaB* and *mtr* which are involved in tryptophan transport across the cytoplasmic membrane; *tnaC* which is required for tryptophan-regulated expression of the *tna* operon, and *trpR* which encodes the tryptophan repressor.

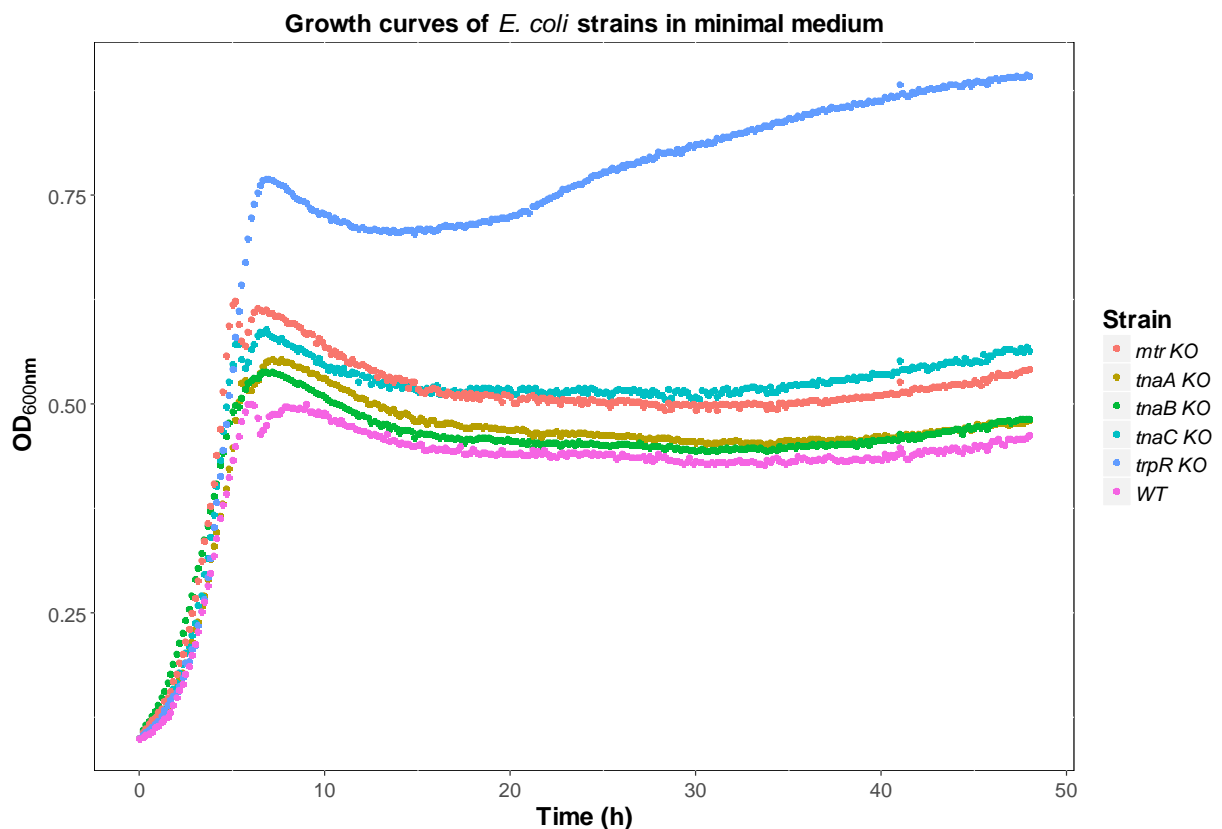


Fig S1. Growth curves of knockout (KO) and wildtype (WT) *E. coli* strains grown in minimal media. Optical density (OD_{600nm}; arbitrary units) *versus* time (h) is shown.

KOVACs test for indole production

KOVACs test is typically used to test for the presence of indole and a color change from clear to pink indicates indole production. This test was performed to test and select *E. coli* KO strains that produce little or no indole. Headspace samples of the selected KO strains were then compared to the wildtype strain to confirm unequivocally that the origin of indole detected in headspace samples is from bacterial production. Presented on the left in Fig S2 are the KO and WT strains grown in minimal medium in the absence of tryptophan, and on the right in the presence of tryptophan. *tnaA* and *trpR* KO strains show little to no change (i.e., they failed to produce significant amounts of indole) and were selected to be compared with the WT strain in headspace experiments.

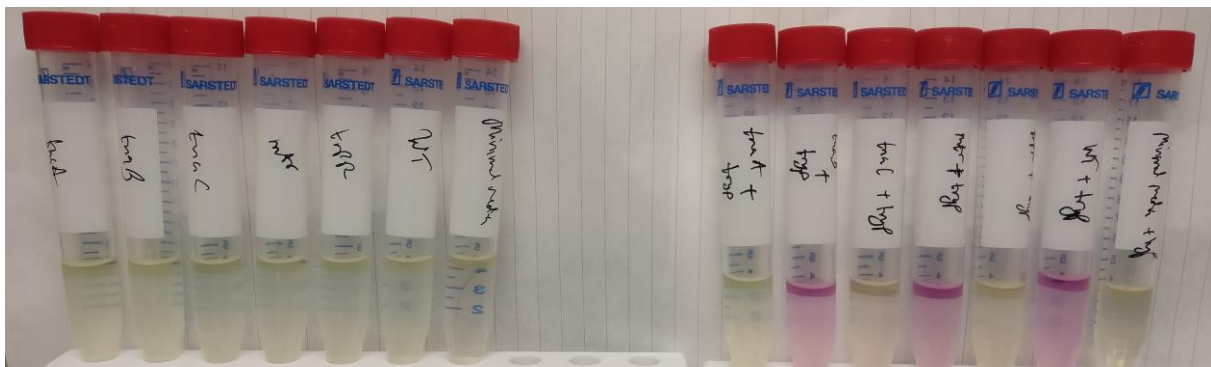


Fig S2. KOVACs test for indole production. Pink appearance indicates indole production.

Growth curves

Growth curves of *P. aeruginosa* grown in the standard and variant forms of artificial sputum medium (ASM) are presented in Fig S3.

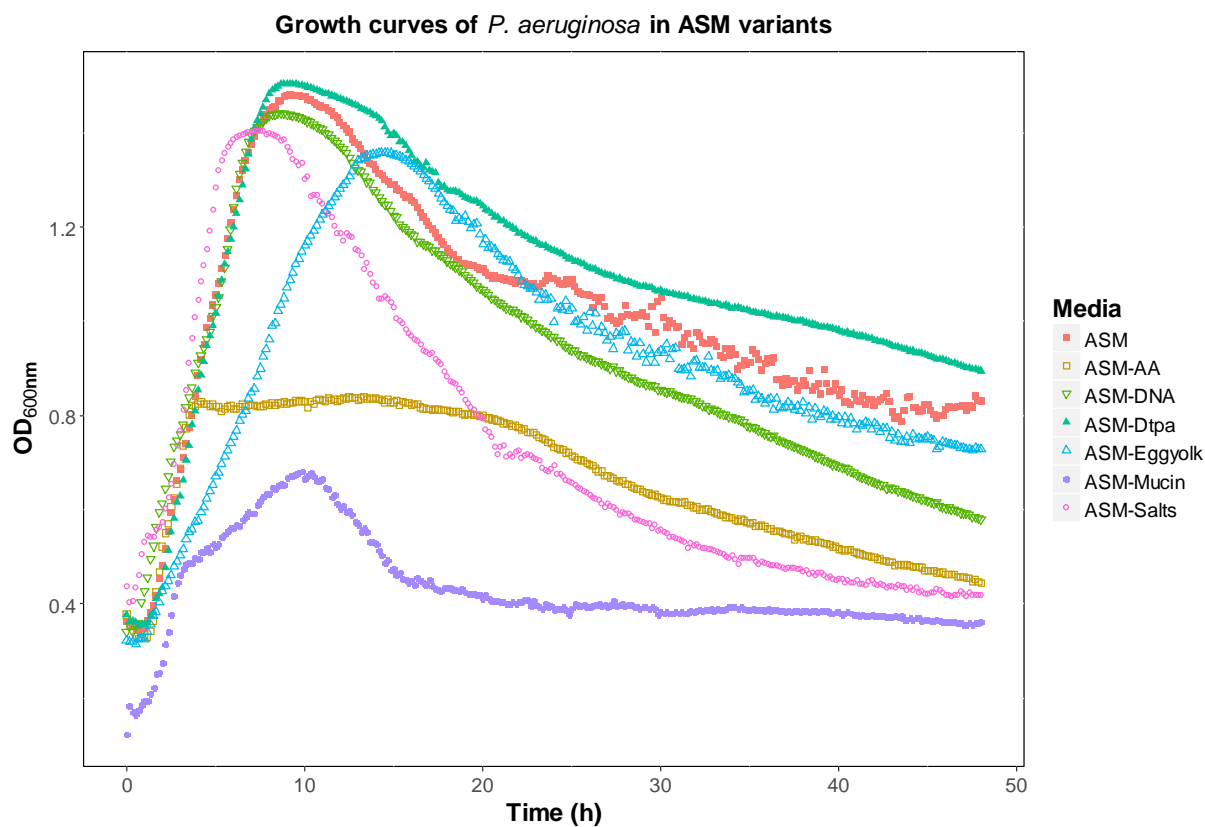


Fig S3. Growth curves of *P. aeruginosa* grown in the standard form and variants of artificial sputum medium (ASM). Optical density (OD_{600nm}; arbitrary units) *versus* time (h) is shown. ASM minus amino acids (ASM-AA), ASM minus deoxyribonucleic acid (ASM-DNA), ASM minus diethylenetriaminepentaacetic acid (ASM-DTPA), ASM minus sodium chloride and potassium chloride (ASM-Salts).

VOC profiling in ASM and NB cultures

Some of the other VOCs observed in the headspace of bacterial cultures are shown in Fig S4.

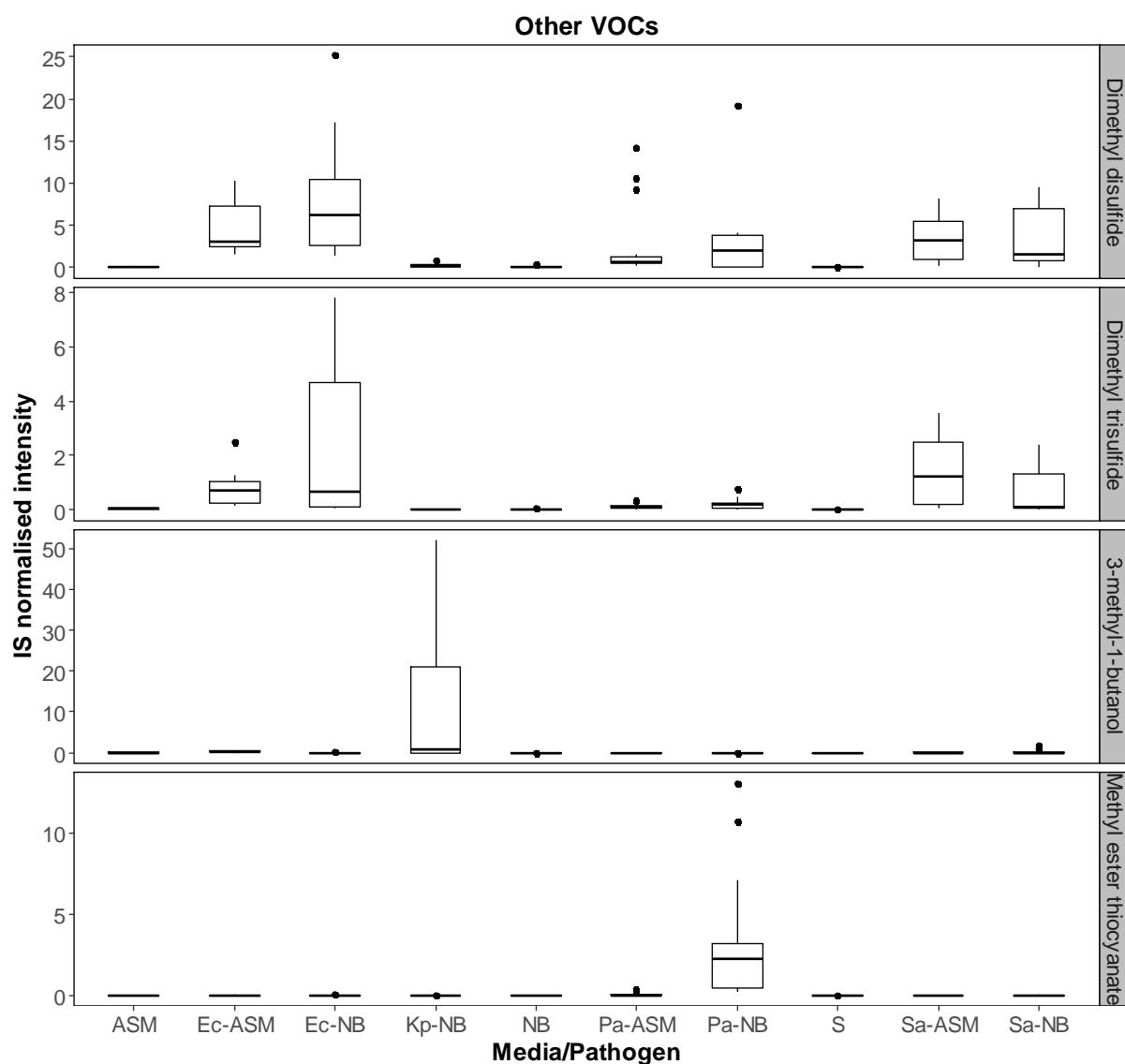


Fig S4. Boxplots showing the internal standard (IS) normalised concentrations of dimethyl disulfide, dimethyl trisulfide, 3-methyl-1-butanol & methyl ester thiocyanate in media only (NB, ASM), bacteria-media conditions (e.g. Ec-ASM), & sorbent only (S). These boxplots are the result of 6 repeat measurements.

Principal component (PC) tuning

Principal component analysis (PCA) was performed on X block data to determine the amount of PCs to include for DFA. Thirty PCs were selected which accounts for approximately 86.9% of variance in the dataset (Figure S5).

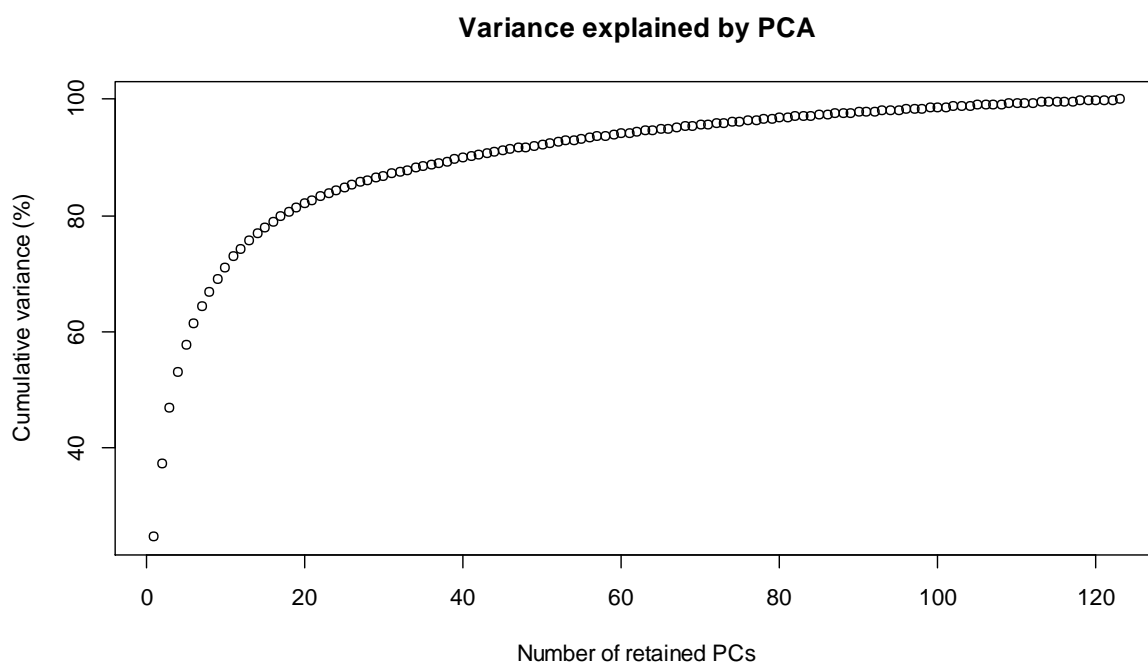
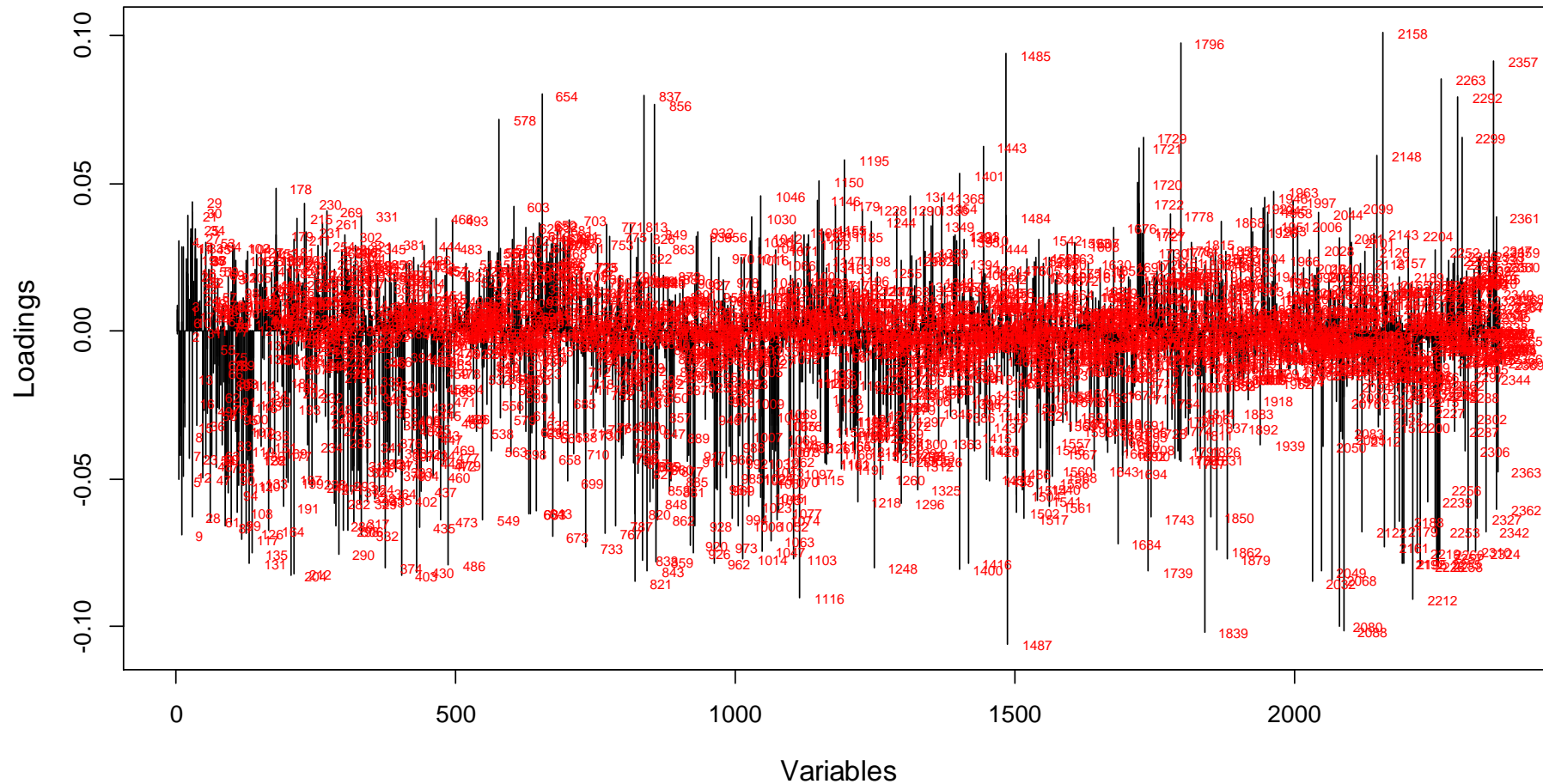


Fig S5. Scree plot output of PCA showing the number of principal components (PCs) extracted and the cumulative percent explained variance.

PC-DFA loadings plot

The variables at the extreme ends of the loading plots were investigated as they mainly contribute most to the separation observed in the scores plot (Fig 5). Fragments 212, 486, 962, & 1014 belong to 1-hexanol and fragments 131, 204, 821, 859 to 2-cyclopenten-1-one (Fig S6). The identity of the other fragments is still unknown.

(a) Loading plot (DF1)



(b) Loading plot (DF2)

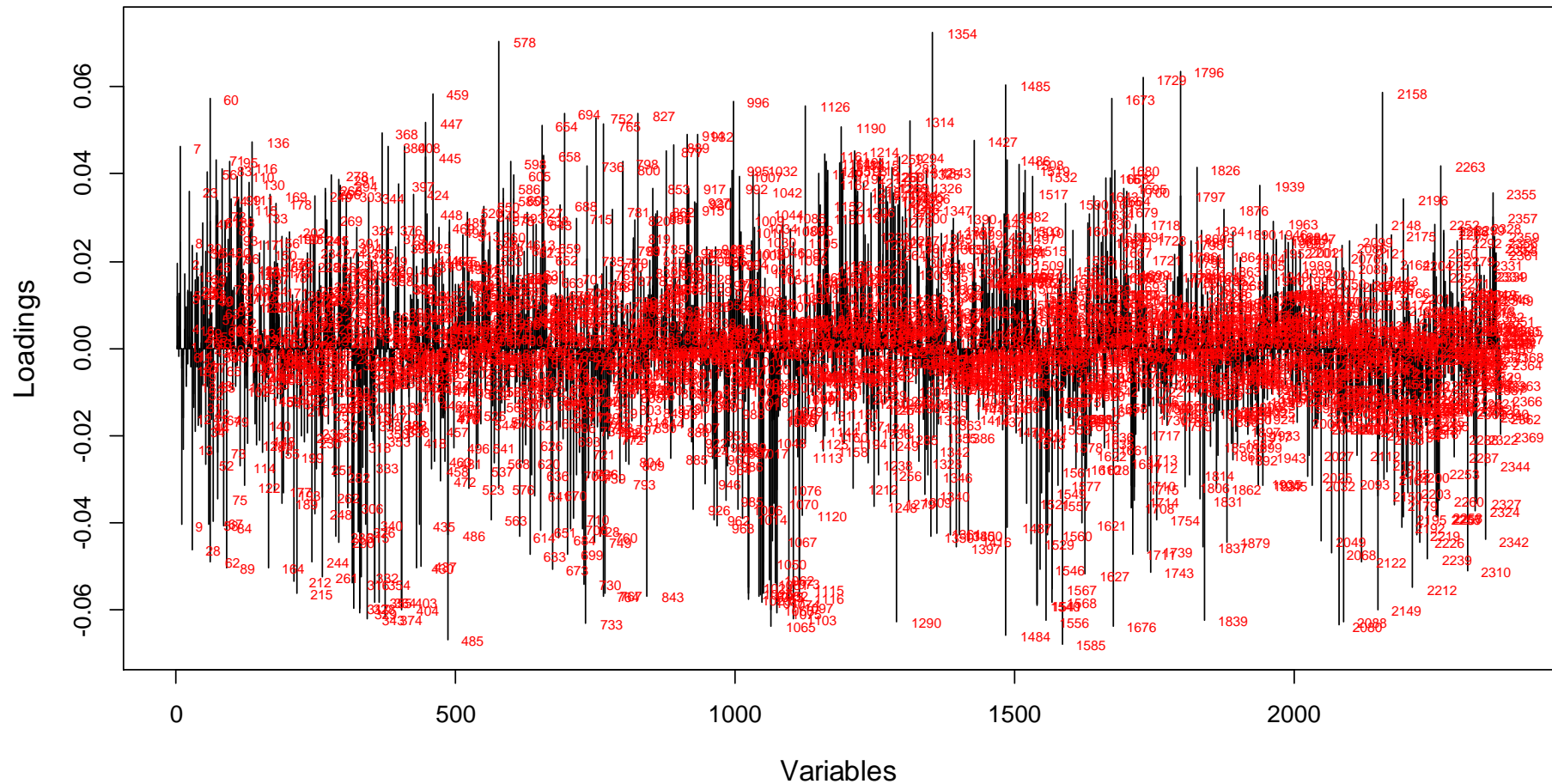


Fig S6. PC-DFA loadings plot for (a) DF1, (b) DF2.