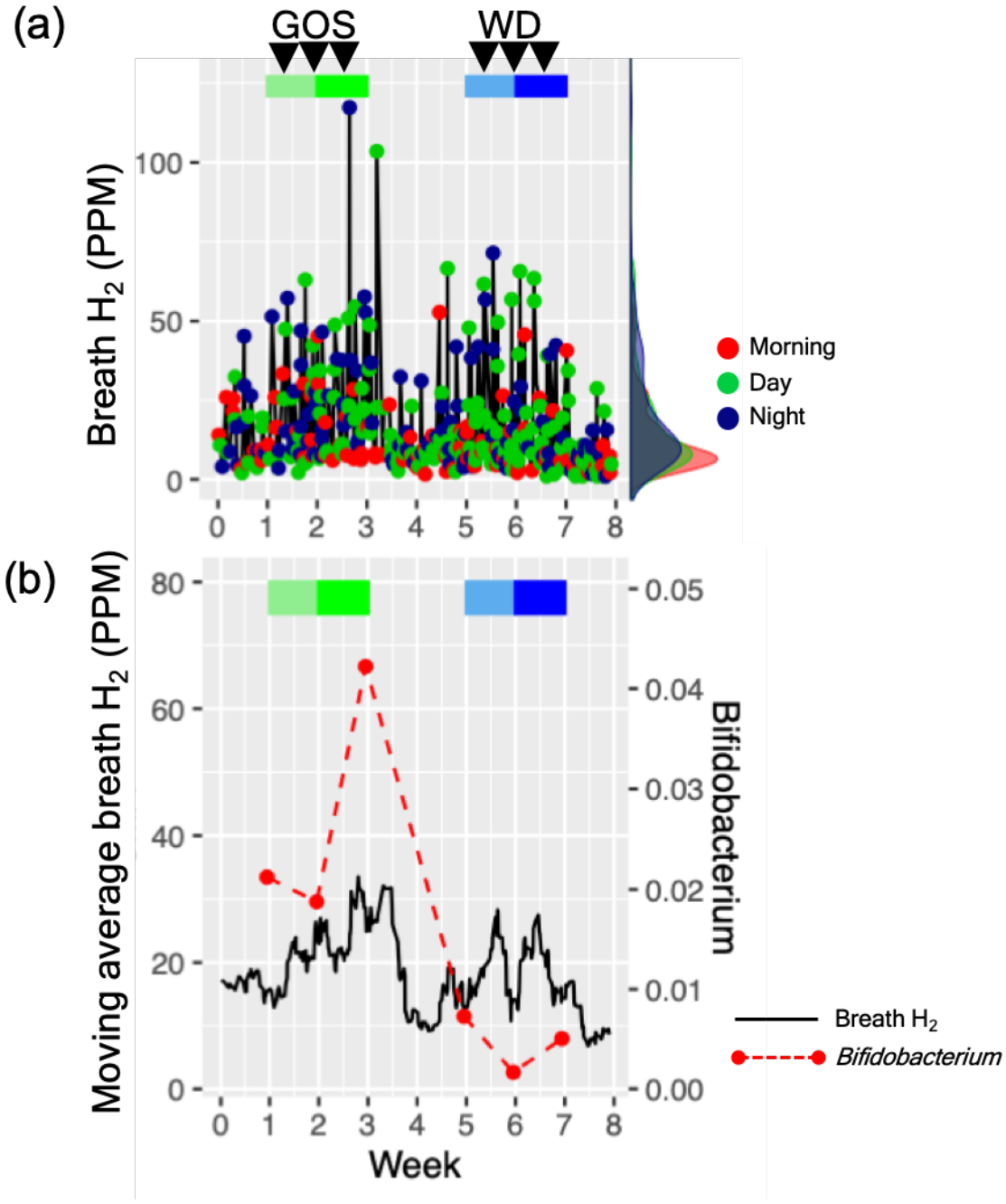
Title: Portable breath hydrogen testing and microbiome analysis identifies prebiotic-induced increases in colonic fermentation and *Bifidobacterium*.

INTRODUCTION: Changes in gut microbiome composition and the attendant health benefits associated with prebiotic intake, vary from person-to-person, implying the need for personalisation in prebiotic treatment. As the primary metabolite of colonic fermentation that can be detected on exhaled breath, hydrogen could be used to indicate when a prebiotic is being metabolized by the host microbiota.

METHODS: Volunteers (n=20) were studied in a double-blind, crossover design (1-week baseline, 2-weeks 1st prebiotic, 2-week wash-out, 2-weeks 2nd prebiotic, 1-week washout) using two different prebiotic fibres, a galacto-oligosaccharide (GOS) and a wheat dextrin (WD). Breath hydrogen scores were recorded using a portable breath analyser, while 6 faecal samples per individual were acquired during the study (2 baseline samples and 4 intervention samples). Bacterial DNA was extracted and submitted to 16S rRNA sequencing on an iSeq platform (Carbiotix) to characterise the gut microbiota.

RESULTS: Five faecal samples were excluded due to low quality DNA, leaving 115 samples. A mean number of 388 breath hydrogen levels were recorded per individual (SD 59). As expected, a high degree of interpersonal variation was apparent in both breath hydrogen profiles and microbiome composition. We noted a consistent trend for increased abundance of the genus *Bifidobacterium* on administration of GOS (group 1–1% (range 0-2.1) to 3.6% (0.4-14.2), p=0.004; group 2–2.4% (0.03-11.6) to 10.2% (0.04-22), p=0.04) and in one of two groups following WD (group 1–0.09% (0-10) to 1.1% (0-19), p=0.04), although this effect was variable between individuals. We also demonstrated an increase in breath hydrogen on administration of GOS but only in the group that received it first in sequence. We next used repeated-measures correlation to correlate the relative abundance of bacterial genera with weekly breath hydrogen levels (calculated using the area under a smoothed curve). Following correction for multiple comparisons, only *Bifidobacterium* was significantly positively correlated with weekly breath hydrogen ($r = 0.35$, adjusted p-value = 0.016).

CONCLUSIONS: These results reflect the known Bifidogenic effects of these prebiotics. Interestingly, *Bifidobacterium* are unable to produce hydrogen, as they do not possess hydrogenases. However, a possible mechanism is that the fermentation of these prebiotics resulted in an initial increase in hydrogen and other important fermentation products, and via microbial cross-feeding, the growth of *Bifidobacterium* was supported to varying degrees in all individuals. However future studies are needed to better understand the processes at play.


(a)
GOS
WD
Breath H_2 (PPM)
100
50
0
0 1 2 3 4 5 6 7 8
Morning
Day
Night
(b)
Moving average breath H_2 (PPM)
80
60
40
20
0
0.05
0.04
0.03
0.02
0.01
0.00
Bifidobacterium
0 1 2 3 4 5 6 7 8
Week
Breath H_2
Bifidobacterium